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Screening and Molecular Characterization of Oleaginous Yeasts and Their Potentiality for Single Cell Oil Production

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Abstract: The study aimed to isolate oleaginous yeasts from different sources (rotten fruits, fruit juices, milk, fish and air) and to identify and to study the possibility of production of single cell oil (SCO) from wheat straw and sugarcane molasses media. Thirty samples of yeasts were isolated using different methods according to the source of isolates. The isolated yeasts were characterized using microscopic appearance, colony morphology, physiological tests, assimilation tests and molecular identification. The effectiveness of oleaginous yeast to produce the single cell oil was studied, by growing the yeast on two types of wheat straw media (detoxified liquid hydrolysate (DLH) and non-detoxified liquid hydrolysate (NDLH)), and molasse media. Single cell oil was extracted by hexane. Eighteen isolates were found to be yeast, and the profile of ten yeast samples was identified as *Saccharomyces cerevisiae*, while, one sample was identified as *Pichia guilliermondii*. DLH wheat straw gave higher productivity of oil than NDLH wheat straw. Generally, *S. cerevisiae* gave higher oil productivity (84 %), compared to *P. guilliermondii* (52 %).

Keywords: Oleaginous yeast, *Saccharomyces cerevisiae*, *Pichia guilliermondii*, single cell oil.

INTRODUCTION

Oleaginous microorganisms are defined as oleaginous species with oil contents excess of 20 % of biomass weight and have fast growth. Most oleaginous yeast can accumulate lipids at levels of more than 40 % of their dry weight and as much as 70 % under nutrient-limiting conditions (Papanikolaou and Aggelis 2011). Nearly 30 known fungal species, mostly yeasts, have been considered as oleaginous microorganisms that have lipid content higher than 20% per dry weight of their biomass. These organisms are not confined taxonomically and phylogenetically to a closely related group. Some are ascomycetes, such as *Lipomyces starkeyi*, *L. lipofer* and *Yarrowia lipolytica*; others are basidiomycetes, such as *Cryptococcus curvatus*, *C. terricola*, *Rhodosporidium toruloides*, *Rhodotorula glutinis* and *R. rubra* (Ratledge and Cohen 2008). Some of oleaginous yeasts have been reported to accumulate lipids up to almost 70 % of their cell dry weight when cultured under nitrogen limited condition. These yeasts belong to genera like *Rhodosporidium*, *Rhodotorula*, *Yarrowia, Candida, Cryptococcus, Trichosporon* and *Lipomyces* (Ageitos *et al.* 2011**)**. The oil produced from those yeasts called single cell oil (SCO). Screening for optimal oleaginous microorganisms became a key mission of many scientists in the field of SCO production (Wu *et al.* 2005). The identification of yeast is based on a combination of morphological and biochemical criteria. The study aimed to isolate oleaginous yeasts from different sources (rotten fruits, fruit juices, milk, fish and air), identify and study the possibility of production of SCO from wheat straw and sugarcane molasses media and to make a comprehensive characterization of the produced oil for further assessment as edible oil.

MATERIALS AND METHODS

Samples were collected from food processing factories, households and local markets. Liquid samples were kept in clean and sterile containers, and solid samples in sterile polyethene bags, then closed and immediately transported to laboratory of the Department of Food Science and Technology of Agriculture, University of Khartoum, Sudan. Oleaginous yeasts were cultured from raw

cow milk, fruit juice (guava, banana and orange), air, fish (obtained from local markets) and rotten fruit (mango, guava, orange and banana).
Molecular analysis of isolated oleaginous yeast was done in the Department of Molecular Parasitology and Medical Entomology, Institute of Endemic Diseases, Faculty of medicine, University of Khartoum. Maxime PCR Premix Kit (i-Taq) was obtained from iNtRON Biotechnology (Korea). Primers were obtained from Macroneg (Korea).

Yeast isolation from Different sources

Oleaginous yeasts were isolated from rotten fruit samples of mango, guava, orange and banana. Yeast isolation from rotten fruit samples was carried out according to the method of Santosh *et al* (2013). Yeast isolation from milk was carried out according to the method of Vasconcelos *et al.* (2019). Yeasts were isolated from air according to the method of Xiaochen *et al.* (2011). Yeasts were isolated from fish according to the method described by Chi *et al.* (2007). Yeast isolation from fruit juice, was done by the method described by Hu *et al.* (2009).

Conventional identification methods

All the strains were identified according to the method described by Barnett *et al*. (2002). Strains from young growing cultures were inoculated into sterile liquid culture media and the culture was microscopically examined after incubation at 28ºCfor three days. The shapes of the strain cells were observed and registered. Also were detected to grow at high concentration of D- glucose and to starch hydrolysis and growth on different temperature range.

Molecular identification

DNA extraction:

DNA extraction was done from yeast cells (1 ml) overnight on YPD agar culture according to Marko and Kersti (2011), with minor modification. Cells were suspended in 200 µl lysis buffer (10 mM Tris, pH 8, mM ethylene diamine tetra acetic acid (EDTA), 100 mM NaCl, 1 % SDS, 2 % Triton X-100) - 300 µl glass bead followed by washing with 4 ml phosphate buffer saline (PBS) until the colour became clear. After each wash a centrifugation was made for 15 minutes at 3000 rpm. After last washing, samples were

transferred to 15 ml falcon tube. One ml white blood cells (WBCs), lysis buffer and 10 µl of 20 mg/µl proteinase K were added, and then samples were incubated at 37°C overnight. On the following day samples were cooled down on the bench at room temperature, then 2 ml of phenol chloroform mixture (25: 24) were added and centrifuged at 3000 rpm for 20 minutes, then 2 ml of pre-chilled chloroform was added and centrifuged at 6000 rpm for 10 minutes, three layers were separated. The supernatant was collected to a new labelled falcon tube; 10 ml of pre- chilled absolute ethanol was added and mixed gently by quickly moving the falcon tube back and forth. Samples were incubated at 20ºC for 24 hours. Then, samples were subjected to quick vortex for 1 min. Samples were centrifuged for 15 min at 3000 rpm and the supernatant was discarded, then samples were washed twice with 4 ml 70 % ethanol and after each wash the supernatant was drained with much care to avoid losing the DNA pellet at the bottom of the falcon tube. The falcon tube was inverted upside down on a tissue paper leaving the pellet to dry from alcohol for at least two hours. Finally, the DNA pellet was re-suspended in 200 µl of deionized water and was incubated at 4ºC for one day to insure complete dissolving of the pellet. Vortexing was applied gently and the DNA was transferred into a new 1.5 Eppendorf tube.

PCR amplification:

The DNA of 13 different yeast isolates were analyzed by polymerase chain reactions using six different primers pairs which were designed according to the isolation, morphological characterization and expected results, to amplify the corresponding gene sequences (Table 1) as described by Joshi and Deshpande *et al.* (2011).

Table 1. PCR primers, sequence and base pair

Primer name	Sequence	base pair
Cryptococcus gatti-F	5′-TGCAAATGCCCATG CATGCT-3′	855
Cryptococcus gatti-R	5′-ATGCGTTCGTCTTC GGCAAA-3′	
Cryptococcus neoformans-F	5′-AATGAAGCGCTCAG GCCAAA-3′	647
Cryptococcus neoformans-R	5′-ATGCGCTGTGCAAA TCACGA-3′	
Saccharomyces cerevisiae-F	5′-AGCGGTTCTGACGT GCAAAT-3′	699
Saccharomyces cerevisiae-R	5′-AGGCCTTTGAAACG GAGCTT-3′	
Pichia guilliermondii–F	5′-ACGACAACGATGTA ACGCCT-3′	666
Pichia guilliermondii–R	5′-TTCGTCACAACGGC CACATA-3′	
Rhodotorula glutinis–F	5′-AACGCAGCGAAATG CGATAC-3′	241
Rhodotorula glutinis–R	5′-ACGCCAAGTCAATC CGAAGT-3′	
Cryptococcus curvatus –F	5′-TTGGACGTTGCGTT CGCATT-3′	628
Cryptococcus curvatus –R	5′-TCTTTGGCTTTGCG GTCTGA-3′	

F: primer forward, R: primer reverse, A: adenine, C: cytosine, G: guanine, T: thiamine

Each isolated strain was subjected to PCR amplification using all the primers to be identified. Special precautions were taken to avoid DNA template or PCR product cross-contamination i.e. DNA extraction and purification, PCR assay, and PCR product electrophoresis were done in separate rooms. The PCR was performed in one step (single tube) in 25 µl final volume using

iNtRON′s Maxime PCR PreMix Kit (i-Tag) according to manufacture instruction. PCR conditions were different according to primers as follow: for *C. gattii* samples were pre-incubated at 95ºC for 1 min, followed by initial denaturation at 95ºC for 5 min, and 35 cycles of denaturation at 94ºC for 1 min, and 35 cycles of annealing at 60ºC for 1 min, and 35 cycles of elongation at 72ºC for 1 min and final elongation at 72ºC for 10 min. For *C. neoformans*; initial denaturation at 95ºC for 10 min, and 30 cycles of denaturation at 94ºC for 1 min, and 30 cycles of annealing at 57ºC for 30 seconds, and 30 cycles of elongation at 72ºC for 1 min and final elongation at 72ºC for 7 min (Leaw *et al.* 2006). For *S. cerevisiae*; samples were pre-incubated at 95ºC for 5 min, followed by initial denaturation at 95ºC for 5 min, and 30 cycles of denaturation at 94ºC for 30 seconds, and 30 cycles of annealing at 55ºC for 30 seconds, and 30 cycles of elongation at 72ºC for 1 min and final elongation at 72ºC for 10 min. For *R. glutinis, P. guilliermondii* and *C. curvatus* primers, PCR condition were as follow: pre- incubated at 95ºC for 2 min, initial denaturation at 95ºC for 10 min, and 30 cycles of denaturation at 94ºC for 50 seconds, and 30 cycles of annealing at 58ºC for 30 seconds, and 30 cycles of elongation at 72ºC for 2 min and final elongation step at 72ºC for 5 min. All PCR conditions were performed on a thermos cycler (Seno Quest brand). As controls, sample of negative (water) and sample of positive (yeast DNA) were included from the beginning of the product in each run according to the primers after PCR amplification according to the method of Joshi and Deshpande (2011). The product was separated on a 1.5 % agarose gel (bio-west) in TBE buffer (89 mM Tri Base, 89 mm Boric Acid, 2 mm EDTA), then was stained with 3 µl ethidium bromide. Five µl of each product was added to 2 µl of loading buffer and then loaded on the gel and left to run in gel documentation system (Bio-RAD Brand) for 90 min at 80 V. The PCR product was visualized by ultraviolet trans-illumination (Bio-Doc-it Brand). Molecular weight of DNA bands was estimated in relation to standard 100 bp DNA molecular weight markers.

Single cell oil production using wheat straw and molasses:

The dilute acid pre-treated hydrolysate and detoxified liquid hydrolysate wheat straw (DLH) were prepared according to the method described by Rosales-Calderon and Aranteso (2019). The detoxified liquid hydrolysate molasses (MDLH) media was prepared using method of Huang *et al*. (2009). The non-detoxified liquid hydrolysate (NDLH) media was prepared according to the method described by Chen *et al*. (2009).

Strain cultivation in NDLH, DLH and MDLH:

Single cell oil producer (yeasts) were grown in the medium containing 3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, and 10 g/L xylose. The medium was first incubated at 30ºC for 24 h as a pre-culturing step. Seed inoculums (10%, v/v) were then added to the culture medium, which included 50 ml each of either (NDLH), (DLH) or (MDLH), as well as 0.4 g/L $MgSO_4,7H_2O$, 2 g/L KH_2PO_4, 0.003 g/L $MnSO_4.H_2O$, 0.0001 g/L $CuSO_4.5H_2O$, and 1.5 g/L yeast extract. Cultures were maintained at 28ºC and 200 rpm in 250 ml flasks (Chen *et al*. 2009).

Dry cell weight determination

To determine the amount of biomass, a 5 ml cell suspension sample was centrifuged at 2500 rpm for 5 min. The cell pellet was then washed twice with distilled water, dried in a pre-weighed aluminium dish at 105ºC for 3 h, and the final mass was expressed as dry cell weight (DCW).

Oil extraction

Yeast cell were harvested from fermentation broth by centrifugation at 8000 rpm for 10 min and frozen overnight, and then oil was extracted by shaking with hexane in separator according to the procedure of Li *et al.* (2010).

RESULTS

Screening and isolation of oleaginous yeast

Table 2 shows the characteristics of isolated oleaginous yeasts colonies and cells and types of vegetative growth. After three days the growth of oleaginous yeasts appeared in all the plates except guava juice samples, as well as one sample of banana juice. The yeast colonies from young growing cultures of rotten fruit were smooth, glabrous, moist and cream. Under the microscope the cells were rod, increase in the vegetative growth was by budding and there were no pseudohyphae except the banana culture where the pseudohyphae were short. Pseudohyphae were clearly found in cultures of air, the colonies were smooth, of mucoid appearance, and pale- pink colour. The cell shape was spherical to ovoid; the vegetative growth was by budding.

Table 2. Characteristics of oleaginous yeasts colonies isolated from different substrates

Sample	Colony character	Cell		
		Shape	Vegetative growth	*Pseudohyphae*
Gf_1	Cream-smooth	Rod	Budding	-
Gf_2	Cream-smooth	Rod	Budding	-
Gf_3	Cream-smooth	Rod	Budding	-
A_1	Cream-smooth	Rod	Budding	-
A_2	Cream-smooth	Rod	Budding	-
A_3	Cream-smooth	Rod	Budding	-
M_1	Cream-smooth	Rod	Budding	-
M_2	Cream-smooth	Rod	Budding	-
M_3	Cream-smooth	Rod	Budding	-
B_1	Cream-smooth	Rod	Budding	+
B_2	Cream-smooth	Rod	Budding	+
B_3	Cream-smooth	Rod	Budding	+
Mi_1	Dark-cream	-	-	-
Mi_2	Dark-cream	-	-	-
Mi_3	Dark-cream	-	-	-
Ai_1	Pale-pink, smooth and mucoid	Spherical to ovoid	Budding	+
Ai_2	Pale-pink, smooth and mucoid	Spherical to ovoid	Budding	+
Ai_3	Pale-pink, smooth and mucoid	Spherical to ovoid	Budding	+
F_1	Cream – smooth and mucoid	-	-	-
F_2	Cream – smoothand mucoid	-	-	-
F_3	Cream – smooth and mucoid	-	-	-
Gj_1	No growth	-	-	-
Gj_2	No growth	-	-	-
Gj_3	No growth	-	-	-
Bj_1	Orange	Ovoid to elongate	Budding	-
Bj_2	Orange	Ovoid to elongate	Budding	-
Bj_3	No growth	-	-	-
Oj_1	Cream to orange	-	-	-
Oj_2	Cream to orange	Rod to ovoid	Budding	-
Oj_3	Cream to orange	-	-	-
Result	Cream-smooth (40%); No growth (23.3%); Pale-pink, smooth and mucoid (16. 7%); Orange (6. 7%); Cream to orange (10%)	Rod (40%); Spherical to ovoid (10%); ovoid to elongate (6. 7%); Rod to avoid (3. 3%); None (40%)	Budding (60%); None (40%)	+ (20%); None (80%)

In this table and the following ones, Gf1\Gf3: isolated from rotten grapefruit, A_1\$A_3$: isolated from rotten apple, M_1\$M_3$: isolated from rotten mango, B_1\$B_3$: isolated from rotten banana, Mi_1\ Mi_3: isolated from milk, Ai_1\$Ai_3$: isolated from air, F_1\$F_3$: isolated from fish, Gj_1\ Gj_3: isolated from guava juice, Bj_1\ Bj_3: isolated from banana juice, Oj_1\$Oj_3$: isolated from orange juice

The fish colonies were cream in colour, smooth and with mucoid appearance. Under the microscope the cell shape was not clear. Microscopic morphology showed ovoid to elongate, budding yeast in banana juice cultures, and the colonies shape was smooth and orange in colour. Orange juice colony shape was glabrous, cream to orange in colour, rod to ovoid shape and budding cell. All the samples grew at 25°C and 37°C, with a weak growth in some isolated samples from rotten mango (M_1, M_2 andM_3), air (Ai_1, Ai_2 andAi_3) at 37ºC. All samples were found to grow at high concentration of D- glucose (Table 3).

Table 3. Physiological and assimilation characteristics of isolated yeast at different temperatures.

Sample	Growth at		Starch hydrolysis
	25°C	37°C	
Gf_1	+	+	+
Gf_2	+	+	+
Gf_3	+	+	+
A_1	+	+	+
A_2	+	+	+
A_3	+	+	+
B_1	+	+	+
B_2	+	+	+
B_3	+	+	+
M_1	+	W*	+
M_2	+	W	+
M_3	+	W	+
Ai_1	+	W	+
Ai_2	+	W	+
Ai_3	+	W	+
Bj_1	+	+	+
Bj_2	+	+	+
Oj_3	+	+	+
Growth (%)	+ (100%)	+ (67. 7%) W (33. 3%)	+ (100%)

*W: weak growth

DNA extracted from isolated yeasts

The quality was measured by Nano-drop spectrophotometer. Purity ranged from 1.8 to 2.1. Total numbers of 13 DNA samples were investigated. Specific primers were used to check if the yeast strains belong to species *C. gatti*, *C. neoformans*, *C. curvatus*, *R. glutinis*, *S. cerevisiae* or *P. guilliermondii*. The selection of these species was based on the results of morphological, physiological characterization, the environment of isolation, specific types of media which used and growing condition. After amplification, the profile of yeast samples isolates from rotten grapefruit (Gf_2), rotten apple (A_1, A_3), rotten mango (M_1, M_3), rotten banana (B_3), air (Ai_2), orange juice (Oj_2) and banana juice (Bj_1,, Bj_2), revealed the presence of specific band 699 bp, and were identical of the profile of *S. cerevisiae*, as indicated in Fig 1. While, a sample isolated from banana (B_2) gave bands of 666 bp, specific to *P. guilliermondii* as indicated in Fig 2. No match profile was found to *C. gatti*, *C. neoformans*, *C. curvatus* and *R. glutinis*. On the other hand, the remaining samples of rotten grapefruit Gf_1 and air Ai_3 were not amplified by all primers.

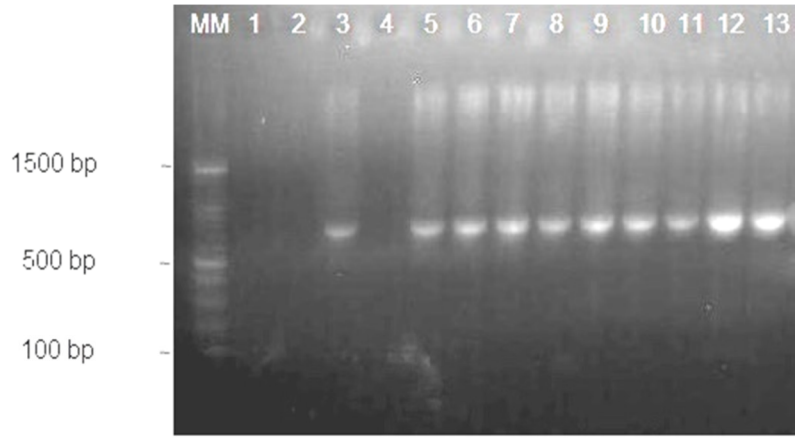

Fig.1 Amplification of 699 bp by PCR for detection of *S. cerevisiae.* Lane M: 100 bp Ladder, lanes 3, 5, 6, 7, 8, 9, 10, 11, 12 and 13 showing positive samples while lane1, 2 and 4 shows negative control result.

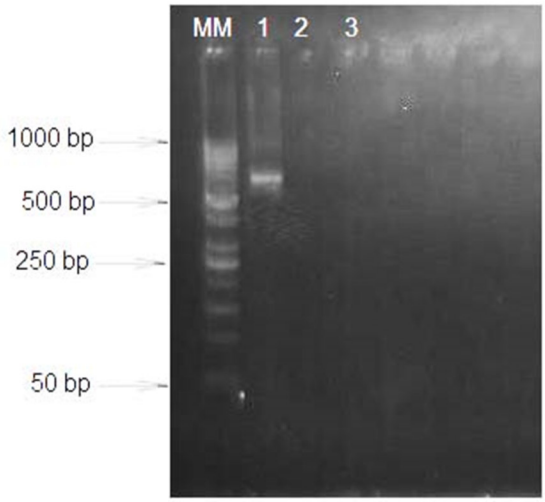

Fig. 2. Amplification of 666 bp by PCR for detection of *P. guilliermondii.* Lane M: 50 bp Ladder, lanes 1 shows positive sample, while lane 2 and 3 showing negative results.

Oil production using wheat straw and molasses liquid hydrolysate substrates

Table 4 shows the highest mean oil % (50.7 %) was found in sample A_1, followed by sample A_3 (48.5 %) in DLH, while the lowest mean value (9.7 %) was found in sample Gf_1 in NDLH.

The highest mean value of biomass 2.6 g/l was detected in the sample Bj_2 and B_3, while the lowest mean value 1.5 g/l was detected in sample A_3. No significant difference ($P \leq 0.05$) in biomass was observed among oleaginous yeast samples under this investigation. The highest oil content was found in sample A_1 (1.5 g/l), while the lowest one was found in sample M_3 (1.0 g), Yeast sample A_3 gave the highest (%) oil production mean value (84.1 %), followed by sample (A_1) (77.8 %). While the lowest one was found in sample Bj_2 (41.7 %). The data showed significant difference ($P \leq 0.05$) among oleaginous yeast samples under this investigation. On DLH media, sample Gf_1

had the lowest (%) oil production (17.9 %). On non- detoxified liquid hydrolysate wheat straw, the oil production of *S. cerevisiae* Oj_2, *S. cerevisiae* Ai_1 and *S. cerevisiae* B_3 were 35. 5 %, 24.8 % and 24.4 %, respectively. The lowest productivity of isolated oleaginous yeast was *S. cerevisiae* M_1.

Table 4. Summary of the similarities and differences between the two single cell oils (wheat straw and molasses) with regard to biomass, oil content (g) and percent (%).

Sample	Biomass (g)			Oil content (g)			Oil (%)		
	DLH	NDLH	Molasses	DLH	NDLH	Molasses	DLH	NDLH	Molasses
M1	1.8^{abcd} ±0.13	1.9^{ab} ±0.02	1.7^{a} ±0.06	0.36^{fghi} ±0.04	0.32^{hij} ±0.01	1.1^{ab} ±0.25	22.2^{fg} ±0.00	16.8^{jkl} ±0.64	67.0^{abc} ±16.69
M3	1.8^{cd} ±0.00	1.9^{a} ±0.00	2.2^{a} ±0.20	0.347^{fgh} ±0.03	0.3^{hi} ±0.01	1.0^{b} ±0.06	20.3^{gh} ±1.41	17.4^{ijk} ±0.35	46.4^{bc} ±6.15
Gf1	1.400^{f} ±0.06	1.8^{cd} ±0.06	2.3^{a} ±1.09	0.3^{ijk} ±0.07	0.2^{k} ±0.01	1.2^{ab} ±0.14	17.9^{hijk} ±3.96	9.7^{n} ±0.71	55.1^{abc} ±19.94
Gf2	1.5^{e} ±0.03	1.8^{bcd} ±0.04	1.6^{a} ±0.14	0.3^{hij} ±0.03	0.3^{ijk} ±0.06	1.1^{ab} ±0.09	20.1^{ghi} ±1.34	14.1^{lm} ±2.90	68.9^{abc} ±0.92
A1	1.3^{g} ±0.01	1.9^{a} ±0.01	2.0^{a} ±0.80	0.7^{a} ±0.03	0.3^{ghi} ±0.00	1.5^{a} ±0.51	50.7^{a} ±1.77	18.6^{hij} ±0.35	77.8^{ab} ±5.59
A3	1.3^{g} ±0.01	1.8^{abc} ±0.03	1.5^{a} ±0.14	0.6^{abc} ±0.01	0.37^{hij} ±0.00	1.3^{ab} ±0.03	48.5^{ab} ±0.28	15.3^{klm} ±0.35	84.1^{a} ±5.73
B2	1.2^{g} ±0.02	1.7^{d} ±0.06	2.4^{a} ±0.48	0.6^{abc} ±0.01	0.3^{jk} ±0.00	1.2^{ab} ±0.14	46.7^{bc} ±1.34	12.8^{m} ±0.14	52.8^{abc} ±16.62
B3	1.240^{g} ±0.00	1.8^{abcd} ±0.01	2.6^{a} ±0.56	0.6^{cd} ±0.00	0.4^{rfg} ±0.00	1.1^{ab} ±0.12	44.3^{cd} ±0.21	24.4^{f} ±0.00	44.7^{bc} ±14.28
Ai1	1.2^{g} ±0.06	1.3^{g} ±0.06	1.7^{a} ±0.35	0.4^{efg} ±0.01	0.3^{hij} ±0.00	0.9^{b} ±0.00	36.4^{e} ±1.13	24.8^{f} ±0.42	58.1^{abc} ±12.16
Ai2	1.3^{g} ±0.01	1.3^{g} ±0.01	1.9^{a} ±0.75	0.3^{hij} ±0.00	0.3^{hij} ±0.01	1.0^{b} ±0.06	23.1^{f} ±0.00	22.7^{fg} ±0.28	56.9^{abc} ±24.96
Bj1	1.3^{g} ±0.03	1.2^{g} ±0.02	2.5^{a} ±0.01	0.5^{def} ±0.00	0.2^{k} ±0.00	1.1^{ab} ±0.19	35.8^{e} ±0.99	14.6^{lm} ±0.14	43.6^{bc} ±7.57
Bj2	1.3^{g} ±0.01	1.2^{g} ±0.06	2.6^{a} ±0.48	0.3^{hij} ±0.21	0.2^{k} ±0.00	1.0^{b} ±0.13	36.5^{e} ±0.00	14.5^{lm} ±0.07	41.7^{c} ±12.66
Oj2	1.5^{ef} ±0.07	1.5^{ef} ±0.02	2.3^{a} ±0.68	0.6^{ab} ±0.05	0.5^{cde} ±0.01	1.0^{b} ±0.11	42.6^{d} ±1.27	35.5^{e} ±0.78	46.6^{bc} ±18.24

Values within columns followed by the same letters are not significantly different at P≤0.05 according to DMRT.

The significantly highest % oil production 84.0 % from molasses was achieved by *S. cerevisiae* A_3 followed by 77.8% A_1 and 67.00 % M_1, while the lowest % oil production 41.7 % was produced by Bj_2. However, the data showed that the *S. cerevisiae* gave higher % oil production of single cell oil than *P. guilliermondii* when using the wheat straw and molasses as substrates.

DISCUSSION

Microscopic morphology lead to the possibility of identification of isolated and screened oleaginous yeasts of rotten grapefruit and rotten banana culture at the genus level *Pichia*, rotten apple and rotten mango culture at the genus level *Saccharomyces*, air culture at the genus level *Cryptococcus* and banana, guava juice culture at the genus level *Rhodotorula*. No match profile was found to *C. gatti, C. neoformans, C. curvatus,* and *R. glutinis*. On the other hand, the remaining samples of rotten grapefruit Gf1 and air Ai3 were not amplified by all primers, due to improper expectation of genus according to isolation. Variation in % oil production depends on the oleaginous yeast samples and DLH, NDLH liquid hydrolysate of wheat straw used in this investigation. The high percentage of oil produced in DLH oil sample indicates the importance of the detoxification process, which might be attributed to that the oleaginous strains were unable to efficiently produce lipids in the presence of the inhibitors in the hydrolysate. So, a detoxification treatment was required prior to the fermentation (Chen *et al*. 2009). These results of oil production were not in agreement with the findings of Xiaochen *et al* (2011), who used this hydrolysate pre-treatment of wheat straw as substrates, their results showed lipid contents of 33.5 % and 27.1 % in the NDLH and DLH, respectively. The biomass of single cell oil on molasses medium ranged from 1.5 to 2.6 g/l which was lower than the findings (8.3 g/L) of Thidarat *et al.* (2012), who used mixed cultures of the oleaginous yeast for microbial oil production using sugar cane molasses as carbon substrate. Results of oil accumulation on molasses medium ranging from 0.9 to 1.5 g were higher than the findings (0.920g) of Thidarat *et al.* (2012). The results of oil production were higher than the findings of Gomes *et al.* (2021), who found that the *S. cerevisiae* strains accumulated more than 40 % of lipids on

sugar cane molasses. The difference was observed in the productivity of single cell oil produced by *S. cerevisiae* based on the different sources of isolation. Compared with some oleaginous yeast and oilseeds productivity, these results were slightly higher than the findings of Sawangkeaw and Ngamprasertsith (2013), who reported the productivity of *Candida curvata* as 29-58 % and *Cryptococcus albidus* as 33-60 %. However, O'Brien (2008), reported the productivity of some oilseeds as Olive (15-35 %), Peanut (45 %), and Sunflower (45 %).

CONCLUSION

- It can be concluded form this study that:
- Oleaginous yeasts can be isolated from rotten fruits, juice, milk, fish, and air, and isolated strains are positive for *S. cerevisiae* and *P. guilliermondii*.
- *S. cerevisiae* are isolated from rotten fruit and fruit juice, while *P. guilliermondii* is isolated from rotten bananas.
- The molasses medium is more effective for the promotion of the accumulation of a substantial amount of lipids by *S. cerevisiae*.
- The productivity of single cell oil produced by *S. cerevisiae* is different according to the sources of isolation.

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عزل الخمائر الزيتية والتعرف علي خصائصها الجزيئية وإمكانية إنتاجها لزيت الخلية الواحدة

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المستخلص: هدفت هذه الدراسة الي عزل الخمائر الزيتيه من مصادر مختلفه (الفواكه المتعفنة،عصير الفواكه، اللبن،السمك والهواء) والتعرف عليها ودراسة إمكانيه إنتاجها لزيت الخليه الواحدة عند زراعتها علي بيئات قش القمح ومولاس قصب السكر. تم عزل ثلاثين عينة وفقا لطرق مختلفة للعزل بناء علي مصدر العزل. وتمت دراسة خصائص الخلايا المجهرية، شكل المستعمرة، الأختبارات الفسيولوجية، الأيضية والجزيئية بعد نموها للتأكد من إنتمائها لعائلة الخميرة. تمت دراسة فعالية الخمائر الزيتية لإنتاج زيت الخلية الواحده، عند نموها على وسط قش القمح NDHL (DHL and) والمولاس. استخلص زيت الخليه الواحده بالهكسان . ووجد أن ثمانية عشر عزلة تنتمي إلى الخميرة وكانت خصائص عشرة عينات متطابقة مع *Sacchromy cerevisiae* بينما، كانت عينة واحدة متطابقة مع *Pichia guilliermondii*. قش القمح DHL أعطي انتاجيه أعلي من قش القمح NDHL. عموما، خميرة *S. cerevisiae* أعطت أعلي انتاجيه للزيت (84 ٪) مقارنة مع خميرة *P. guilliermondii* (52 ٪).