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Lipolytic Mycoflora In Fatura

Joheni Mohamed Jwely and Mohamed Ahmed Al-Ryani

Department of Botany, Faculty of Science, Zintan University

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*Corresponding author E-mail**:** profdrnani@uoz.edu.ly (Mohamed A. Al-Ryani)

Abstract \

The current study was aimed for isolation, identification and preservation of mycobiota associated with the olive oil processing wastes (Fatura) collected from different cities in Al-Gabal Al-Gharby, Libya, screening the cold-active lipolytic activity of the isolated fungi and selection of the highest cold-active lipase producers. 31 fungal species belong to 12 genera were isolated from these samples with total CFUs of 29560.*Fusarium* was the most common genus at total CFUs of 9020 and comprising 30.51% from all fungi, followed by *Aspergillus*, that recorded 25.44% from all fungi. *Penicillium* was ranked third, nine different species were present. A total of 100% of samples were found to have CFUs of 5140 and 17.4% of all fungi. On lipase production agar medium at two temperatures, 10 and 20°C, 102 fungal isolates from 31 species were tested for their lipolytic activity. The majority of fungi could produce lipase activity at 20°C, where 98 out of 102 isolates the highest lipase producers was higher at 10°C (25) than at 20°C (16). The most active isolates were *Alternaria*, *Fusarium*, and *Penicillium*. Molecular identification of the most active four isolates was carried out by sequencing their internal transcribed spacer region (ITS).

Keyword: Olive oil, cold active enzymes, Lipase, fungi, lipolytic activity, Fatura.

Introduction \

Table olives and olive oil are characteristic Mediterranean products with recognized economic and dietary benefits. Eating table olives and olive oil provides a range of health benefits, including a lower risk of heart disease and various types of cancer, according to numerous studies. These findings have prompted extensive research into the components of olive fruits and the kinds of olive fruit components that are accountable for the observed beneficial health effects [45, 3, 16]. Oil content significantly affects lipase production. It was discovered that utilizing 2% of olive oil as an enhancer led to a maximum lipase activity of around 30.30 U/g starting dry weight after 24 hours of cultivation, as opposed to peptone's activity of 27.80 U/g initial dry weight [5]. *Rhizopus oligosporous* GCBR-3 strain was shown to have a maximum activity of 302.1U/g in earlier experiments, and employing *Rhizopus* strains also increased activity [44, 28]. Among the numerous types of microbes, fungi and yeast are recognized as possible sources of fungal lipase. Fungi produce extracellular lipolytic enzymes that are simple to extract and purify, which lowers production costs and makes them the preferred source over bacterial lipases. Fungal lipases are substrate-specific and stable under a wide range of chemical and physical conditions [43, 30]. The most common fungal strains today produce commercial lipases in their culture medium, including; *Candida rugosa*, *Rhizopus oryzae*, *Mucor miehei*, *Rhizopus japonicus*, *Rhizopus arrhizus*, *Rhizopus delemar*, *Rhizopus niveus*, *Aspergillus niger*, and *Thermomyces lanuginosus*. Commercial lipases derived from fungi are used in a variety of industrial fields, including; production of detergents, food and dairy products, pharmaceuticals and medicine, biodiesel, oleochemicals, leather industry, also used in wastewater bioremediation, cosmetics and perfumeries, ester synthesis, paper manufacturing, and bioremediation [6. 23]. Due to their selectivity and advantages for future development, fungal lipases have enormous promise as biocatalysts for the creation of biomolecules [29, 17, 26]. The following are the most significant advantages: they have the high efficacy under mild reaction conditions, easier to practice in the natural reaction medium and products, capable to decrease contamination from the environment, accessibility of lipases from diverse fungal sources, and enhancement of catalytic power of lipases through genetic engineering. The current study aimed to isolation, identification and preservation of mycobiota associated with the olive oil processing wastes (Fatura) collected from different cities in Al-Gabal Al-Gharby, Libya, screening the cold-active lipolytic activity of the

isolated fungi and selection of the highest cold-active lipase producers.

Materials and methods \

Collection of Fatura samples: In Libya during the season 2022, seven composite samples of the waste products from the manufacturing of olive oil (Fatura) were gathered from Zintan, Rayaina, Jadu, Yafran, Rujban, Al-Asabaa, and Gharyan. Samples were put in sterile plastic bags and brought right to the mycological lab, at science college of Zintan university for further studies.

Isolation and preservation media: Czapek's Dox Agar(CYA)was used as an isolation medium, and Potato Dextrose Agar (PDA) were used for preservation of the isolated fungi. The dilution plate technique [47] was used for isolation. In this method, 20 grams of each sample were individually placed in Erlenmeyer conical flask contains 80ml sterile distilled water and was shacked for 60 min at 150 rpm to make a spore suspension from the sample. 1mL of the spore suspension was separately transferred to sterile Petri plates that containing medium growth. and incubated at 25°C for 10 days. Five plates were used for each sample. The fungal that appeared was purified on Cz-agar medium. Colony forming units (CFUs) of each fungal species was calculated according to the following Equation:

$$\text{Colony forming units (CFUs)} = \frac{\text{Total count of each fungal species x Dilution factor}}{\text{Number of plates for the sample}}$$

Identification of fungi: The identified up to species level using culture and morphological features under the light microscopic following the keys and descriptions of [13, 36, 39, 5, 25, 33, 11].

Preservation of the isolated fungi: All the isolated fungi were preserved in the culture collection of the Assiut University Mycological Centre (AUMC) using three different methods of preservation, namely on PDA slants at 4°C [42, 34], in 15% glycerol water (Glycerol, 15mL and distilled water, 85mL) at -80°C [12, 42], and as lyophilized ampoules [42, 13].

Screening of lipolytic activity: The medium described by [46] was used for screening the lipolytic activity of the isolated fungi. The medium composed of (g/L): Peptone, 10; $MgSO_4$ $.7H_2O$, 0.2; $CaCI_2.2H_2O$, 0.2; Tween 80, 10 ml; and agar, 20. The medium was dispensed aseptically in 15-cm test tubes (12 ml/tube), then inoculated by 50 µL of spore suspension from 7-day-old cultures of tested fungi. The tubes were then incubated at 10 and 20ºC for 14 days. The lipolytic ability was observed as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. The depth of each visible precipitate (in mm) was measured.

Molecular identification of the potent fungi:

DNA extraction: A small amount of 7-day-old fungal cultures grown on PDA was separately scraped and suspended in 200 µl of sterile distilled water in 2ml sterile vials and boiled at 100°C for 15 minutes [10]. An 800µl CTAB buffer composed of 3% CTAB, 1.4 M NaCl, 0.2% Mercaptoethanol, 20mM EDTA, 100mM TRIS-HCl pH 8.0 and 1% PVP-40, were added to each tube. After incubation at 65°C for 30min, 800µl of CI Mix with the composition of 24ml chloroform and 1ml isoamyl alcohol, were gently added and mixed with the tube contents. A clear supernatant was obtained by centrifugation at 10000xg for 10min. For DNA precipitation 2/3 volume of isopropanol (precooled at -20°C) was added and mixed gently. The samples were incubated at 4°C overnight, thereafter centrifugation at 13000xg for 10min. The supernatant was discarded and the pellet was pooled and washed with 200µl washing buffer composed of 76% ethanol and 10mM ammonium acetate. The washing buffer was carefully decanted and the pellet was suspended in 200µl TE buffer supplemented with 10mg/ml RNase. After incubation at 37 °C for 30min, 100µl of 7.5M ammonium acetate and 750µl ethanol were added and mixed gently. Samples were centrifuged at 13000xg for 10min at room temperature. The supernatant was completely discarded and the pellet was suspended in 100µl sterile distilled water.

PCR for rDNA and sequencing using ITS1 and ITS4 primers: The universal primers ITS1 and ITS4[48] were used for DNA amplification. In the PCR tubes 1µl of DNA template, 1µl 2.5mM dNTP mix, 0.2unit of Taq polymerase, 5µl of 10x complete buffer and 40µl of sterile ddH_2O, 10pmol of ITS1 (5´ TCC GTA GGT GAA CCT TGC GG 3´) and ITS4 (5´ TCC TCC GCT TAT TGA TAT GC 3´) were added. Then the PCR amplification was carried out using the following sequence: one round of amplification consisting of denaturation at 95°C for 15 min followed by 30 cycles of denaturation at 95°C for 20sec, annealing at 50°C for 40 sec and extension at 72°C for 1min, with a final extension step of 72°C for 5min. The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. The purified PCR products were confirmed on 1% agarose gel by electrophoresis using size marker. The bands were eluted and sequenced in the forward and reverse directions.

Alignment and phylogenetic analysis: Contiguous sequences of the fungal species in this study were uploaded to GenBank and accession numbers were given. Sequences of the nearest species were downloaded from GenBank including sequences of the available type specimens. All sequences in this analysis were

aligned together using MAFFT [22] with the default options. Alignment gaps and parsimony uninformative characters were optimized by BMGE [8]. Maximum-likelihood (ML) and Maximum parsimony (MP) phylogenetic analyses were performed using MEGA X version 10.2.6[24]. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications [15]. The best optimal model of nucleotide substitution for the ML analyses was determined using Akaike information criterion (AIC) as implemented in Modeltest 3.7[37]. The phylogenetic tree was drawn and visualized using MEGAX [31]. The resulting tree was edited using Microsoft Power Point (2016) and saved as TIF file [2].

Results \

Fungi recovered from Fatura: In this study, seven composite samples of olive oil processing wastes (Fatura) were obtained from Zintan, Rayaina, Jadu, Yafran, Rujban, Al-Asabaa, and Gharyan. 31 fungal species belong to 12 genera were isolated from these samples with total CFUs of 29560. *Fusarium* was the most common genus encountering total CFUs of 9020 and comprising 30.50% of total fungi. It was represented by two species, *F. oxysporum* and *F. solani*. *F. solani*as illustrated in (Figure 1), was the most prevalent comprising 94% of total *Fusarium* and 28.7% of total fungi, while *F. oxysporum* encountered approximately 6.0% of total *Fusarium* and 1.8% of total fungi. *Fusarium* was followed by *Aspergillus*, which made up 25.44% of all fungi and was represented by eight species. It appeared in all samples, which had a total CFU count of 7520.With 29.25% of all *Aspergillus* and 7.4% of all fungi, *Aspergillus aureolatus* was the most common species (Table 1). *Penicillium* was ranked third. Nine different species were present. A total of 100% of samples were found to have CFUs of 5140 and 17.4% of all fungi. *Penicillium aurantiogriseum* was the species that was found the most frequently across 6 of the 7 samples. 5.8% of all fungi and 33.85% of all *Penicillium* were present in it. The fourth most prevalent genus, which made up 14.14% of all fungi, was *Mucor*, which was represented by two known and one undetermined species. 4180 CFUs were discovered. The most common species, accounting for 88% of all *Mucor* and 11.9% of all fungi, was *M. hiemalis*. Below is a description of the study locations and isolated fungi:

Fungi isolated from Zintan: Eleven species related to seven genera comprising CFUs of 5700 were identified from Zintan city at 25°C. Genus *Aspergillus* wasincluded 3 species (*A. aureolatus*, *A. niger* and *A. ustus*). It encountered 26% (1500 CFUs) of total counts of all fungi isolated. From these species, *A. niger* yielded the highest number of propagules (580 CFUs) followed by *A. ustus* (520) and *A. aureolatus*

(400). *Penicillium* and *Alternaria* (2 species for each one), were the runner of *Aspergillus*. *Penicillium* (11.6 %) was represented by *P. roquefortii* (10.5%) and *P. citrinum* (1%). *Alternaria* constituting 5.6% (320 CFUs) of total fungi. *A. alstroemeriae* and *A. angustiovoidea* as shown in (plates; 1, 2) (200 and 120 CFUs) respectively. On the other hand, the genus *Fusarium* was represented by (*F. solani*) comprising the highest number of propagules (35.4% of total fungi). The remaining genera, *Microdochium*, *Mucor* and *Phialophora* were represented by one species only.

Fungi isolated from Rayaina: Five genera represented by thirteen species were recovered from this sample. Species richness was pronounced in both *Aspergillus* and *Penicillium*, they were represented each by 4 species. The genus *Aspergillus* showed the highest CFUs (2020 colony out of 4260). The most common species was recovered in high CFUs was *A. fumigatus*. *Penicillium* follows *Aspergillus* in the number of colonies (780 out of 4260 colony). It was represented by *P. aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum* and *P. citrinum* (constituting 6.1 of total count of *Penicillium*). The results also showed that genus *Mucor* was represented by 3 species (*M. hiemalis*, *M. racemosus* and *Mucor* sp.) and harbored 12.2% of total count. Whereas *Fusarium* (*F. solani*) and *Stachybotrys* (*S. chartarum*) were recovered with one species each as shown.

Fungi isolated from Jadu: Four genera and 10 species were collected from Jadu in this investigation. Noticeably, genus *Penicillium* harbored the highest number of species (5) and total count of colony (2180 out of 5480, 39.8%). The genus represented by *P. aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum*, *P. citrinum* and *P. puberulum* with the most common was *P. aurantiogriseum*. *Aspergillus* (1120 CFUs, 20.4% of total count) was reported in two species *A. aureolatus* (20.4%) and *A. deflectus* (14.6%). In addition, *Fusarium* was represented by two species (*F. oxysporum* and *F. solani*), it was constituting high number of colonies (36.6% of total fungal count in Jadu). The fourth genus, *Phialophora* was isolated as *P. richardisae* (3.3% of total CFUs).

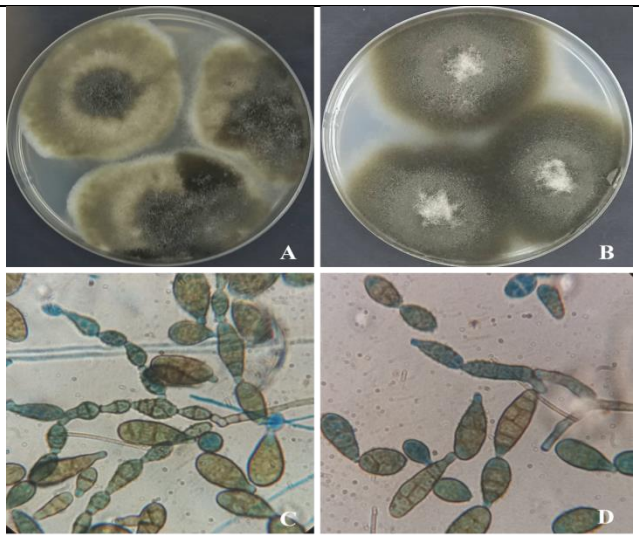
Fungi isolated from Yafran: Nine species related to seven genera were reported here. Only genus *Penicillium* reported in 3 species (*P. aurantiogriseum*, *P. chrysogenum* and *P. expansum*) with 12.1% of total fungal colonies. The other remaining genera were represented by only one species. Regarding to the number of colonies forming units, *Mucor hiemalis* showed the highest (30.5%) followed by *Fusarium solani* (25.9%). The other species ranged from 2.8 to 14.2% of total count.

Fungi isolated from Rujban: Six genera presented by 11 species were recovered, regarding the number of species, genus *Penicillium* was the first. It was reported in 4 species with *P. crustosum*(Figure 1), is the most common (140 colony out of 400 *Penicillium* colonies). It is worth mentioning that, genus *Fusarium* (*F. oxysporum* and *F. solani*) was recovered in high CFUs (1960, 45% of total count). *Aspergillus* was the same in the number of species (*A. aureolatus* and *A. niger*), both species harbored 13.8% of total count. The other remaining genera (3) were presented in one species each (*Geotrichum candidum*, *Mucor hiemalis* and *Trichoderma harzianum*) and CFUs ranging from 5.5 to 15.6% of the fungal count percentage.

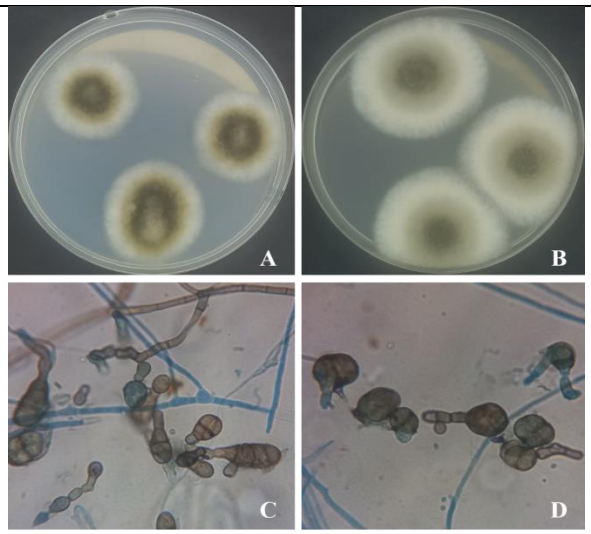
Fungi isolated from Al-Asabaa: Four genera and 8 species were encountered in this sample. The most common genus was *Fusarium*, it was showed the highest count 40.7% of total fungal count (700 colonies out of 1720) and represented by 2 species (*F. oxysporum* and *F. solani*). *Aspergillus* follows *Fusarium* in number of colonies (represented by 2 species), it was encountered 29.1% of the total count. As shown in table (1), *Penicillium* was the most diverse genus, it was represented by 3 species. It was harbored 15.1% of total fungal count**.**

Fungi isolated from Gharyan: Five genera including 11 species were totally collected from this sample. The most diverse genus was *Aspergillus*, recovered in 6 species, giving rise 55.8% of the total fungi. *A. fumigatus* the most common species, it was presented in 16.7% of *Aspergillus* count. *Penicillium* was reported in two species, *P. aurantiogriseum* and *P. griseofulvum*, giving rise 7.5% of the total fungi. The remaining species; *Fusarium solani*, *Mucor hiemalis* and *Myrothecium verrucaria* were emerged in 20.83, 8.34 and 7.5% of the total fungi.

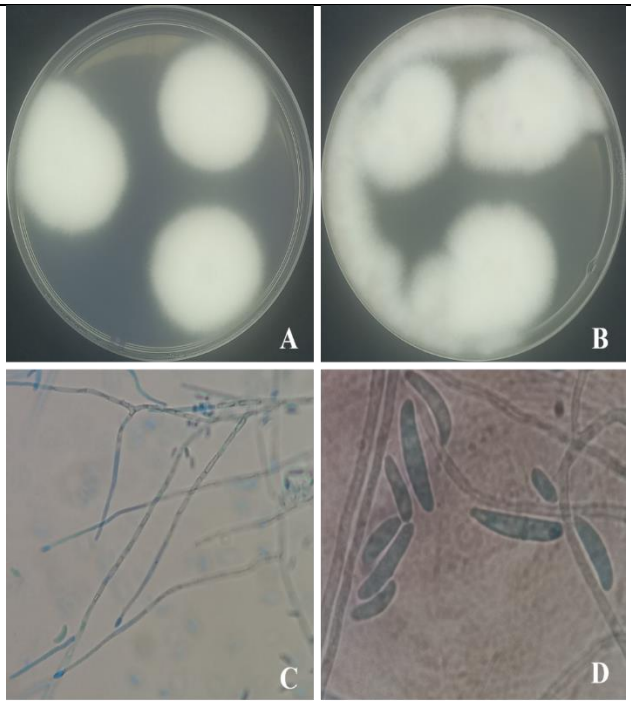
Screening of lipolytic activity: On lipase production agar medium at 10 and 20°C, 102 fungal isolates from 31 species related to 12 genera were tested for their lipolytic activity. The majority of fungi could produce lipase activity at 20°C, where 98 out of 102 isolates, were able to do so, compared to 73 isolates at 10°C, while the number of intermediate lipase producers was wider at 20°C (71) than at 10°C (29), the number of the highest lipase producers was higher at 10°C (25) than at 20°C (16). *Aspergillus* species (8) were completely positive at 20°C compared to 4positive species at 10°C, all *Penicillium* species (9) demonstrated positive findings at examined, 10 and 20°C (Table 2).



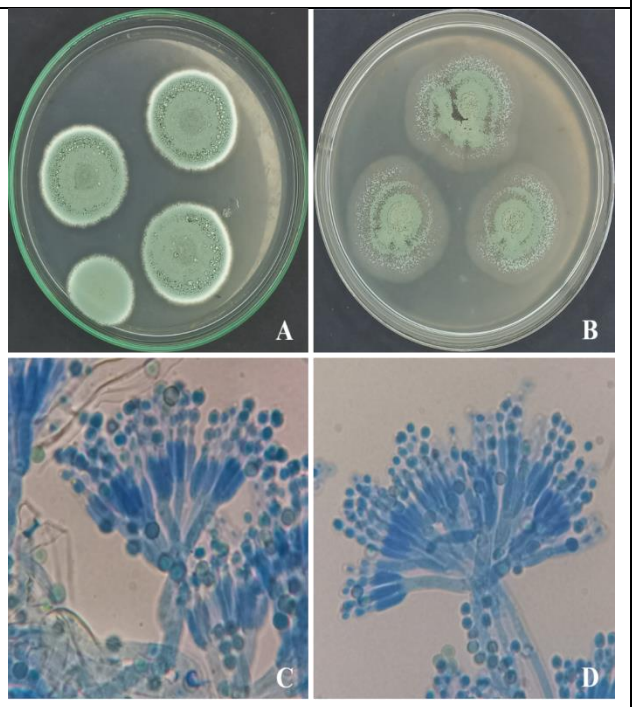
Plate;1. ***Alternaria alstroemeriae*****AUMC 16060:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, chains of conidia.



Plate;2. ***Alternaria angustiovoidea*** **AUMC 16062:**A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, chains of conidia.



Plate;3. ***Fusarium solani*****:** A-B, Seven-day-old colonies on Cz and PDA at 25°C. C, Long phialides bearing microconidia. D, Falcate macroconidia.



Plate;4. ***Penicillium crustosum*****:** A-B, Seven-day-old colonies on Cz and MEA at 25°C. C-D, Conidiophores and terverticillate penicilli.

Figure 1: Morphological identification for important lipolytic fungi isolates.

Table 1. CFUs (Colony forming units) and CFUs% of fungi recovered from different samples of olive oil processing wastes (Fatura).

Fungal species	Zintan		Rayaina		Jadu		Yafran		Rujban		Al-Asabaa		Gharyan		Gross total	
	CFU	**%**	**CFU**	**%**	**CFU**	**%**	**CFU**	**%**	**CFU**	**%**	**CFU**	**%**	**CFU**	**%**	**CFU**	**%CFU**
***Alternaria* spp.**	**320**	**5.61**													**320**	**1.1**
A. alstroemeriae	200	3.51													200	0.7
A. angustiovoidea	120	2.1													120	0.4
***Aspergillus* spp.**	**1500**	**26.3**	**2020**	**47.42**	**1120**	**20.4**	**440**	**7.8**	**600**	**13.8**	**500**	**29.1**	**1340**	**55.83**	**7520**	**25.44**
A. aureolatus	400	7.0	600	14.1	320	5.8	440	7.8	200	4.6			240	10	2200	7.4
A. caespitosus											200	11.2	220	9.2	420	1.4
A. deflectus			120	2.8	800	14.6									920	3.1
A. fumigates			800	18.8									400	16.7	1200	4.0
A. niger	580	10.2							400	9.2			140	5.83	1120	3.8
A. silvaticus													280	11.6	280	0.94
A. terreus											300	17.4			300	1.0
A. ustus	520	9.1	500	11.7									60	2.5	1080	3.65
***Fusarium* spp.**	**2020**	**35.44**	**380**	**8.91**	**2000**	**36.5**	**1460**	**25.9**	**1960**	**44.9**	**700**	**40.7**	**500**	**20.83**	**9020**	**30.5**
F. oxysporum					120	2.2			160	3.6	260	15.1			540	1.8
F. solani	2020	35.4	380	8.91	1880	34.3	1460	25.9	1800	41.3	440	25.6	500	20.83	8480	28.7
Geotrichum candidum									**240**	**5.5**					**240**	**0.8**
Humicola fusco-atra							**800**	**14.2**							**800**	**2.7**
Microdochium nivale	**120**	**2.11**													**120**	**0.4**
***Mucor* spp.**	**880**	**15.44**	**520**	**12.21**			**1720**	**30.5**	**680**	**15.6**			**380**	**15.84**	**4180**	**14.14**
M. hiemalis	880	15.44	40	0.94			1720	30.5	680	15.6			200	8.34	3520	11.9
M. racemosus			360	8.45											360	1.2
Mucor sp.			120	2.8											120	0.4
Myrothecium verrucaria													**180**	**7.5**	**180**	**0.6**

***Penicillium* spp.**	**660**	**11.6**	**780**	**18.31**	**2180**	**39.8**	**680**	**12.1**	**400**	**9.2**	**260**	**15.1**	**180**	**7.5**	**5140**	**17.4**
P. aurantiogriseum			180	4.2	1000	18.2	280	4.9	100	2.3	120	7.0	60	2.5	1740	5.8
P. brevicompactum			160	3.75	120	2.2									280	0.94
P. chrysogenum			180	4.2	600	10.9	200	3.6			100	5.8			1080	3.65
P. citrinum	60	1.0	260	6.1	260	4.7			80	1.8	40	2.3			700	2.36
P. crustosum									140	3.2					140	0.47
P. expansum							200	3.6							200	0.67
P. griseofulvum									80	1.8			120	5	200	0.67
P. puberulum					200	3.6									200	0.67
P. roqueforti	600	10.5													600	2.0
Phialophora richardisae	**200**	**3.5**			**180**	**3.3**	**160**	**2.8**							**540**	**1.82**
Stachybotrys chartarum			**560**	**13.15**											**560**	**1.9**
Trichoderma harzianum							**380**	**6.7**	**480**	**11.0**	**260**	**15.1**			**1120**	**3.8**
Total	**5700**	**100**	**4260**	**100**	**5480**	**100**	**5640**	**100**	**4360**	**100**	**1720**	**100**	**2400**	**100**	**29560**	**100**
No. of genera	**7**		**5**		**4**		**7**		**6**		**4**		**5**		**12**	
No. of species	**11**		**13**		**10**		**9**		**11**		**8**		**11**		**31**	

Table 2. Preliminary screening of lipolytic activity of fungi isolated from olive oil processing wastes (Fatura) at 10 and 20°C on sucrose-free Cz agar supplemented with 1% tween 80.

Fungal species	No. of isolates	At 10 °C				At 20 °C			
		Positive	H	M	L	Positive	H	M	L
Alternaria	**2**	**2**	**2**			**2**		**2**	
A. alstroemeriae	1	1	1			1		1	
A. angustiovoidea	1	1	1			1		1	
Aspergillus									
A. aureolatus	6	2	1		1	5	5		
A. caespitosus	3					3	1	2	
A. deflectus	2	1			1	2		2	
A. fumigates	1					1		1	
A. niger	4	1			1	4		3	1
A. silvaticus	1					1	1		
A. terreus	3	2			2	3		3	
A. ustus	2					2	1	1	
Fusarium									
F. oxysporum	5	5	4	1		5		5	
F. solani	19	19	13	5	1	19	3	16	
Geotrichum candidum	1	1		1		1		1	
Humicola fusco-atra	1	1			1	1			1
Microdochium nivale	1	1			1	1			1
Mucor									
M. hiemalis	7	7		7		7	1	4	2
M. racemosus	2	2			2	2		1	1
Mucor sp.	1	1		1		1		1	
Myrothecium verrucaria	1	1	1			1	1		
Penicillium									
P. aurantiogriseum	10	2	1	1		10		10	
P. brevicompactum	2	2		1	1	2		2	
P. chrysogenum	6	6		3	3	6		4	2
P. citrinum	7	6		5	1	6		6	
P. crustosum	1	1		1		1		1	
P. expansum	1	1		1		1		1	

Fungal species	No. of isolates	At 10 °C				At 20 °C			
		Positive	H	M	L	Positive	H	M	L
P. griseofulvum	2	2		1	1	2		2	
P. puberulum	1	1		1		1		1	
P. roquefortii	1	1	1			1	1		
Phialophora richardisae	3					1			1
Stachybotrys chartarum	2	1			1	2			2
Trichoderma harzianum	4	4	2		2	4	2	2	
Total	**102**	**73**	**25**	**29**	**19**	**98**	**16**	**71**	**11**
No. of genera	**12**	**11**	**6**	**4**	**8**	**12**	**6**	**7**	**7**
No. of species	**31**	**26**	**9**	**13**	**14**	**31**	**9**	**23**	**8**

H= highest lipolytic activity (more than or equal 11mm), M= intermediate (from 5mm to less than 11mm), and L= low (less than 5mm)

The most active isolates were *Alternaria* (2 isolates), *Fusarium* (1), and *Penicillium* (1). Molecular identification of the most active four isolates was carried out by sequencing their internal transcribed spacer region (ITS). Pure cultures of the fungal isolates were deposited in the culture collection of Assiut University Mycological Centre as *Alternaria* sp. AUMC 16060, *Alternaria* sp. AUMC 16062, *Fusarium* sp. AUMC 16063, and *Penicillium* sp. AUMC 16061.

Molecular identification of the potent lipase producers:as shown in Figures; (2, 3,4,5).

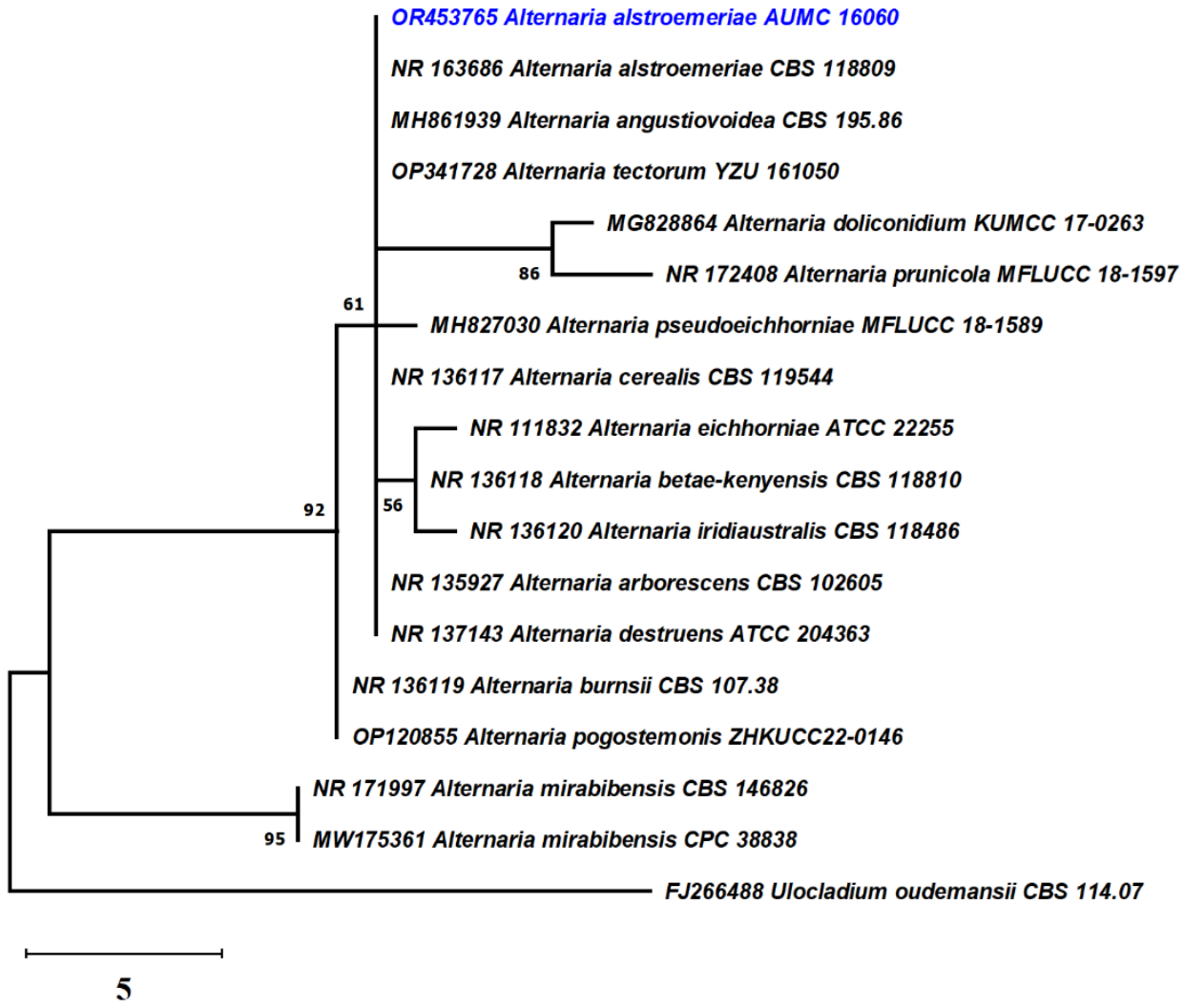


Figure 2. The most parsimonious tree obtained from a heuristic search (1000 replications) of *Alternaria alstroemeriae* AUMC 16060's ITS sequence (in blue color) compared to other closely similar ITS sequences belonging to *Alternaria* in GenBank. Bootstrap support values are indicated near the respective nodes. The tree is rooted to *Ulocladium oudemansii* CBS 114.07 as out group.

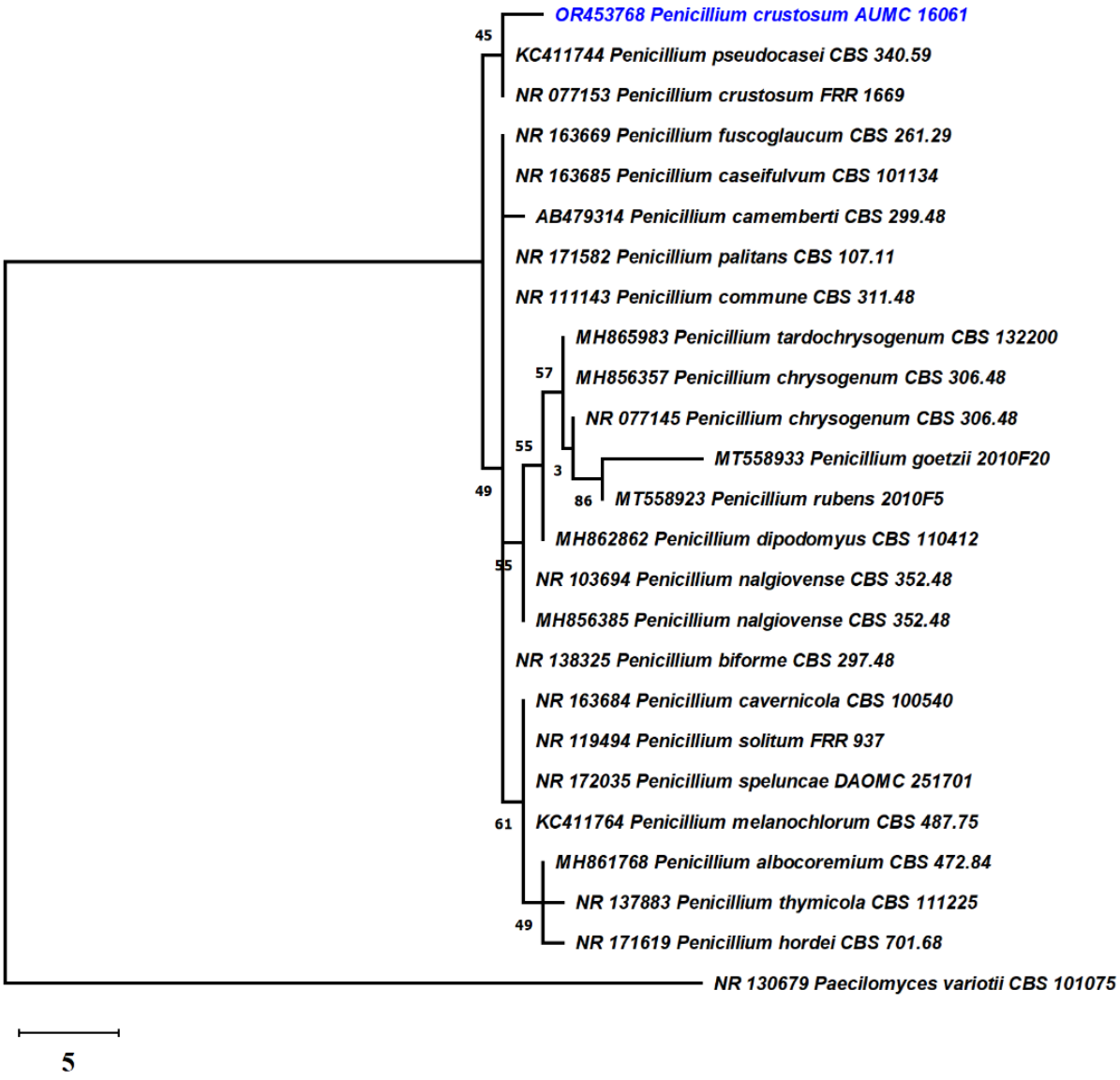


Figure 3. The most parsimonious tree obtained from a heuristic search (1000 replications) of *Penicillium crustosum* AUMC 16061's ITS sequence (in blue color) compared to other closely similar ITS sequences belonging to genus *Penicillium* in GenBank. Bootstrap support values are indicated near the respective nodes. The tree is rooted to *Paecilomyces variotii* CBS 101075 as out group.

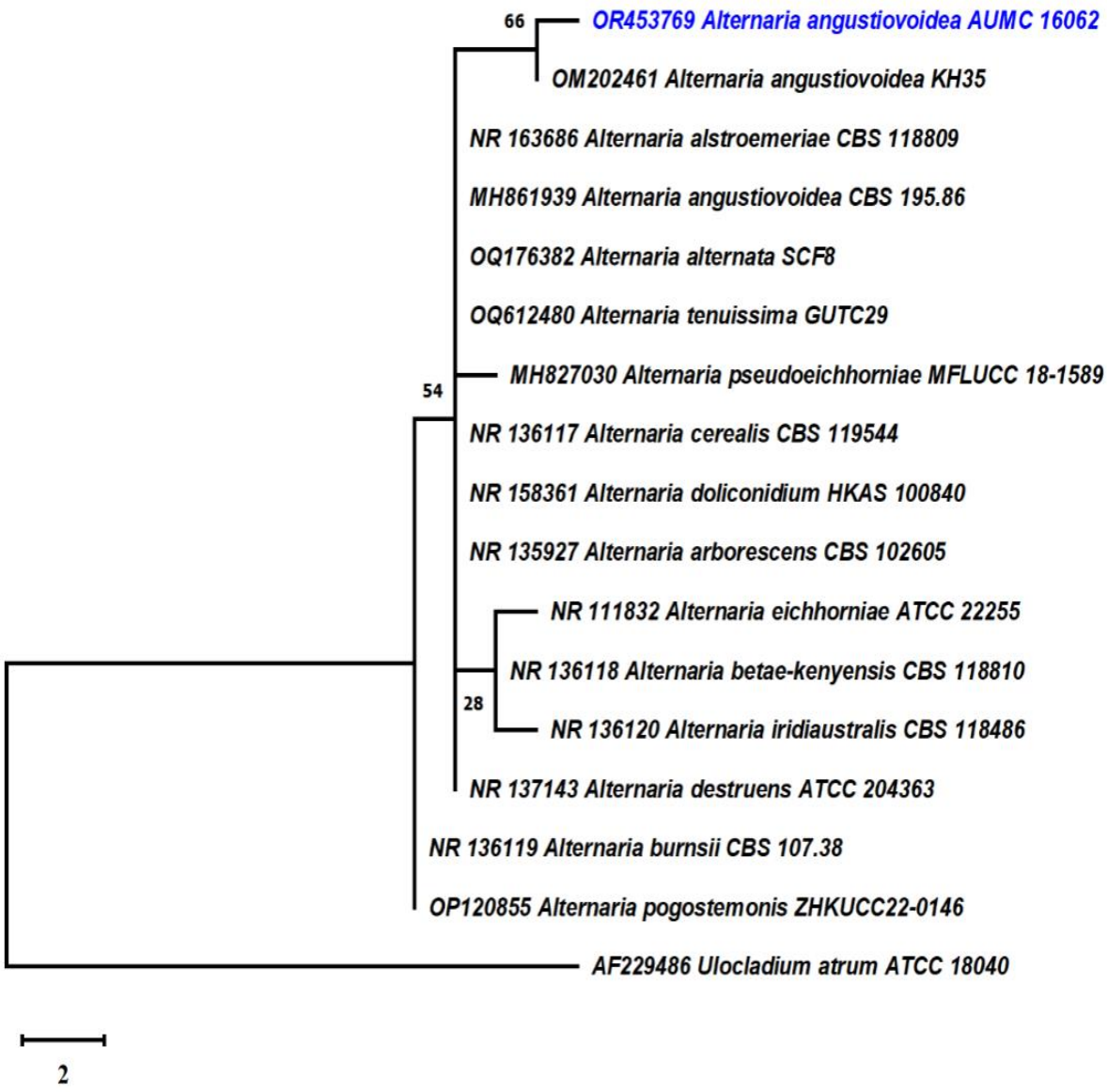


Figure 4. The most parsimonious tree obtained from a heuristic search (1000 replications) of *Alternaria angustiovoidea* AUMC 16062's ITS sequence (in blue color) compared to other closely similar ITS sequences belonging to *Alternaria* in GenBank. Bootstrap support values are indicated near the respective nodes. The tree is rooted to *Ulocladium atrum* ATCC 18040 as out group.

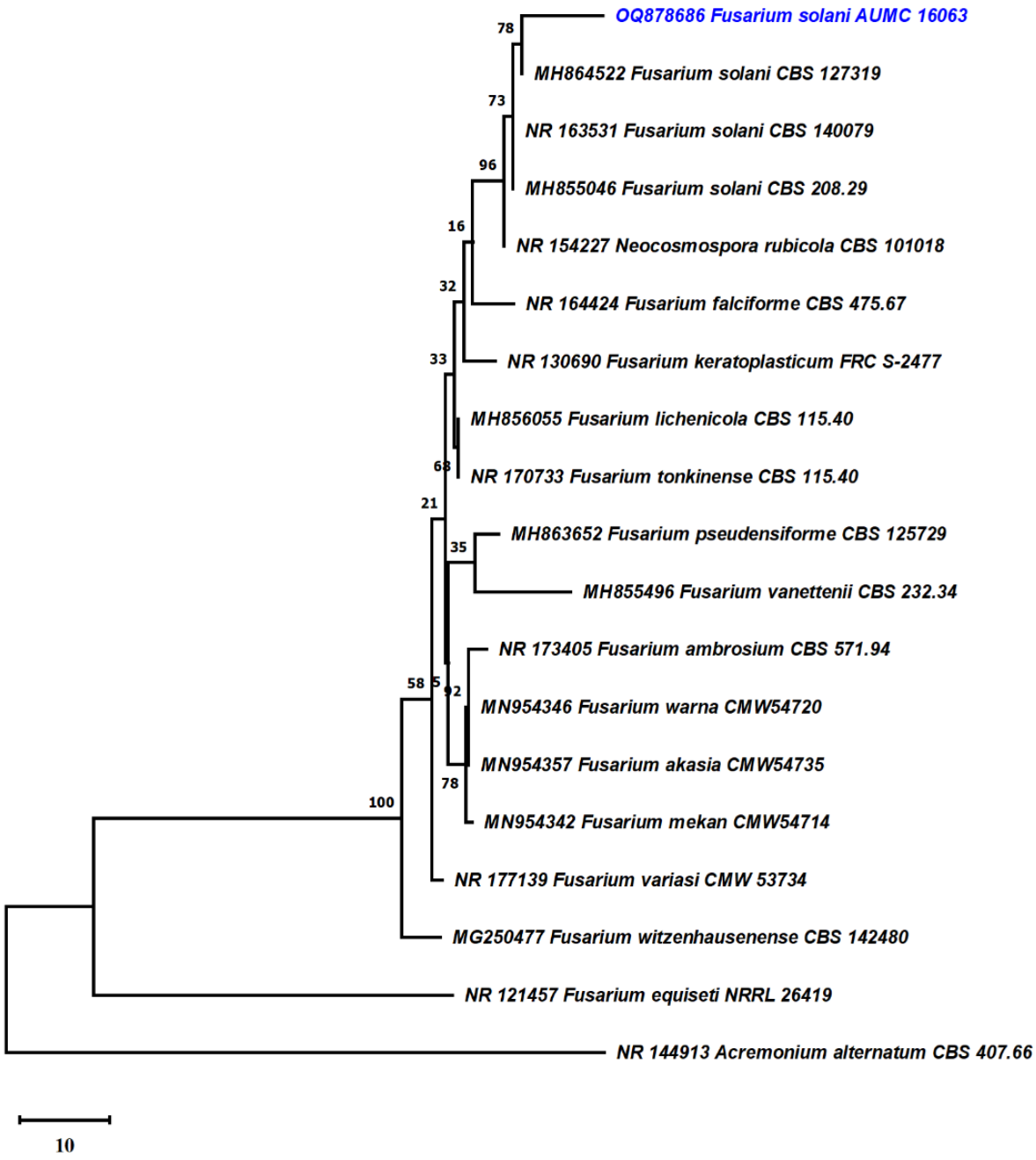


Figure 5. The most parsimonious tree obtained from a heuristic search (1000 replications) of *Fusarium solani* AUMC 16063's ITS sequence (in blue color) compared to other closely similar ITS sequences belonging to *Fusarium* in GenBank. Bootstrap support values are indicated near the respective nodes. The tree is rooted to *Acremonium alternatum* CBS 407.66 as out group.

Discussion \

The current study was aimed to identify the fungi that were present in seven composite samples of olive oil processing wastes (Fatura) that were collected from Libyan different cities include; Zintan, Rayaina, Jadu, Yafran, Rujban, Al-Asabaa, and Gharyan. One of the most important duties to comprehend any bioremediation process is monitoring microbial communities. Only a few research concentrating for identification of microbial communities in olive mill wastes have been carried out, despite the fact that the necessity of monitoring microbial diversity has been widely discussed. Indeed, such research projects make it possible to analyze the biotransformation of olive mill waste in detail. To the best of our knowledge, this study is the first to focus on the isolation of fungi from Fatura and their usage in Libya to create cold-active lipases. The literature on biodiversity studies on the worldwide isolation of fungi from Fatura is very sparse. In this study, 31 fungal species from 12 genera were isolated from these samples. *Fusarium* was the most common genus encountering 30.51% of total fungi. It was represented by two species, *F. oxysporum* and *F. solani*. Several studies recorded that the incidence of fungisuch as *Acremonium*, *Alternaria*, *Aspergillus*, *Chalara*, *Fusarium*, *Lecythophora*, *Paecilomyces*, *Penicillium*, *Phoma*, *Phycomyces*, *Rhinocladiella*, and *Scopulariopsis*in olive mill [49, 32, 40]. *Pichia* (*P. guilliermondii*–syn. *Meyerozyma-guilliermondii*) and *Candida* (*C. diddensiae* and *C. ernobii*)spp., were also the main yeast biota in OMW from Moroccan olive mills[4].*Pichia caribbica* (syn. *Meyerozyma caribbica), P. holstii* (syn. *Nakazawaeaholstii),* and *Zygosaccharomyces fermentati (*syn. *Lachancea fermentati*) were the predominant yeast taxa in two-phase olive mill waste(TPOMW), while *Z. florentinus* (syn. *Zygotorulaspora florentina*), *Lachancea thermotolerans* (syn. *Kluyveromyces thermotolerans*), *Saccharomyces cerevisiae*, and *S. rosinii* (syn. *Kazachstaniarosinii*) were minor constituents of the yeast community[41]. Some of the yeast isolates from two-phase olive mill waste (TPOMW) exhibited cellulase, *β*-glucanase, *β*-glucosidase, peroxidase, and poly-galacturonase activities which could contribute to the degradation of complex compounds, including olive pomance phenolics. Based on the data provided by [41], yeast diversity in olive pomance appears to be variety dependent. A search of the National Center for Biotechnology Information (NCBI) turned up 106 deposited

sequences of fungi found in waste environments at olive mills. The majority of the fungal species from olive mill wastes are classified as Glomeromycota, Basidiomycota, Ascomycota, and unclassified fungi, according to analysis of these sequences. More than 60% of the fungal species stored in GenBank are occupied by members of the Basidiomycota. Members of the Glomeromycota and unclassified fungi make up 19 and 17% of the records, respectively, while Ascomycota makes up just 3% of the total number of representatives. Since primers unique to Basidiomycota and Glomeromycota were used for the majority of the detected species, the Ascomycota population is actually underestimated [43, 44].

The potential to detoxify olive mill effluents has been found in members of the fungal genera *Acremonium*, *Alternaria*, *Aspergillus*, *Chalara*, *Fusarium*, *Lecythophora*, *Paecilomyces*, *Penicillium*, *Phoma*, *Phycomyces*, *Rhinocladiella*, and *Scopulariopsis* in OMW disposal ponds [31]. However, rather than using molecular methods, these fungi were identified based on their morphology. Native microbiota that may break down OMW phenolics include those belonging to the fungal genera *Cerrena*, *Byssochlamys* (syn. *Paecilomyces*), *Lasiodiplodia*, and *Bionectria*, which were discovered using molecular methods [20]. *Pichia*, *Candida*, and *Saccharomyces*-like species are the most common yeasts in olive mill wastes, according to the studies cited above. Yeasts in olive mill wastes primarily act metabolically by reducing phenolics and sugars, albeit they appear to contribute less to OMW decolorization than white-rot fungi [19]. Additionally, the waste from olive mills may have an advantage over bacteria due to their acidic pH. While white-rot fungi have been isolated to a lesser extent, filamentous fungus, such as *Aspergillus* and *Penicillium* spp., are frequent inhabitants of olive mill wastes [31, 7]. It appears that the high levels of salt and sugar in olive mill wastes, along with the waste's acidic pH, encourage the establishment of osmotolerant yeasts in olive mill wastes [18].

On lipase production agar medium at 10 and 20°C, 102 fungal isolates from 31 species related to 12 genera were tested for their lipolytic activity. The majority of fungi could produce lipase activity at 20°C, more than at 10°C, but while the intermediate lipase producers was the best at 20°C than at 10°C, while the highest lipase producers was a higher at 10°C than at 20°C. All *Aspergillus* species were completely positive at 20°C except to 4 positive species at 10°C, where all

Penicillium species demonstrated positive findings at both 10 and 20°C. These results were agreed with [50, 9, 7, 20, 49].

The understanding of cold-active lipases is expanding quickly, however research on cold active lipases is fragmented and lacking. No initiatives have been made to date to arrange this data. As a result, from the material found in the literature, an overview of this crucial enzyme for biotechnology and industry as well as its traits has been collected. It is evident from the scant reports on cold active lipases that have been published that the majority of research on these enzymes has been devoted to their isolation, purification, and characterization.

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الأحياء الفطرية المحبة للدهون في الفيتورة

جهينة محمد جويلي و محمد أحمد الرياني

قسم علم النبات ، كلية العلوم، جامعة الزنتان.

Corresponding author email: profdrnani@uoz.edu.ly

المستخلص /

هدفت الدراسة الحالية إلى عزل وتشخيص وحفظ الأحياء الفطرية المرتبطة بمخلفات معالجة زيت الزيتون (الفيتورة)، جمعت العينات من مدن مختلفة في الجبل الغربي-ليبيا، وتم فحص نشاط التحلل الدهني البارد النشط للفطريات المعزولة واختيار أعلى منتجي الليباز البارد النشط، عزل 31 نوعاً فطرياً تنتمي إلى 12 جنساً من هذه العينات بإجمالي وحدات مستعمرة 29560، الفيوزاريوم هو الجنس الأكثر شيوعاً بمجموع وحدات مستعمرة 9020، وشكل 30.51% من مجموع الفطريات، يليه جنس السبيروجلس الذي سجل 25.44% من مجموع الفطريات، واحتل البنسيليوم المرتبة الثالثة، متضمناً تسعة أنواع مختلفة، تواجدت في إجمالي 100% من العينات بإجمالي تردد 5140 وحدة مكونة لمستعمرة (CFU)، وبنسبة 17.4% من مجموع الفطريات، في وسط أجار إنتاج الليباز عند درجتي حرارة 10 و 20°م، تم اختبار 102 عزلة فطرية من 31 نوعا لنشاطها في التحلل الدهني، تمكنت غالبية الفطريات من إنتاج الليباز النشاط عند 20 درجة مئوية، حيث كانت 98 منتجة من أصل 102 عزلة، وكان أعلى إنتاج عند 10°م(25عزلة) مقارنة بدرجة 20°م (16عزلة)، وكانت العزلات الأكثر نشاطا؛ ألترناريا، والفيوزاريوم، والبنسليوم، تم إجراء التحديد الجزيئي للعزلات الأربع الأكثر نشاطًا من خلال تحديد تسلسل منطقة المباعد الداخلي (ITS).

الكلمات الدالة: زيت الزيتون، الإنزيمات النشطة الباردة، الليباز، الفطريات، نشاط التحلل الدهني، الفيتورة.