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Isolation and Molecular Identification of Lipase-Producing Yeasts from Marine and Oil-Contaminated Environments in Basra, Iraq

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I. Abstract

Lipases are hydrolytic enzymes with important applications in biotechnology, food processing, biodiesel production, and environmental remediation. This study aimed to isolate and identify lipase-producing yeasts from environmental samples collected from marine and oil-contaminated sites in Basra, Iraq. A total of 80 soil and water samples were collected from different locations, including oil-contaminated soils, seawater, pond and swamp water, and agricultural soils. Yeast isolates were purified and initially characterized using macroscopic and microscopic features, followed by preliminary biochemical identification using the VITEK-2 system. Molecular identification was performed by PCR amplification and sequencing of the internal transcribed spacer *ITS* region of ribosomal DNA.

ITS-based analysis confirmed three lipase-producing yeast isolates belonging to two species: *Cyberlindnera jadinii* and *Candida parapsilosis WISP1* and *Candida parapsilosis WISP3*. The isolates showed positive lipolytic activity on Spirit Blue Agar supplemented with olive oil, as indicated by clear zones around the colonies. Lipase activity was further supported using modified Rhodamine B olive oil agar, where orange fluorescent halos were observed under ultraviolet light. Sudan Black B staining also indicated intracellular lipid accumulation in the tested yeast isolates under nitrogen-limited and carbon-rich conditions. These findings provide preliminary qualitative evidence that selected environmental samples from Basra contain yeast isolates capable of producing extracellular lipase under the tested screening conditions. Further quantitative enzymatic assays would be required to evaluate lipase production efficiency.

Keywords:

C. jadinii, C. parapsilosis, Enzymatic activity, Lipase enzyme, Lipase-producing yeast, Modified Rhodamine B olive oil agar

II. Introduction

Lipase is a type of hydrolase enzyme (EC 3.1.1.3) which is an effective enzyme for breakdown of triglycerides into fatty acids and glycerol in the presence of water and oil [1, 2]. Lipases are of paramount importance in food, industrial and technological applications because of their ease of production, possibilities for genetic modification and high stability [3]. These enzymes have been extracted from a variety of sources such as animals, plants, yeasts, fungi, and bacteria. This variety of source of lipases gives them their versatility and high biological distribution. One of the most important functions of these enzymes is their use for the treatment of fatty waste, which is converted into economical compounds and so helps reduce pollution and leads to the better use of organic materials [4]. Lipases find extensive applications in the food industry, including dairy products, where

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they are employed in the modification of fats for example, to improve the taste or texture of cheese or to break down fat in milk. They are also applied in the production of detergents, chemical reactions such as transesterification, biofuels and biomedicine, as well as the production of biodegradable materials and polymers [5-7]. Microbial lipases (obtained from bacteria, yeasts or fungi)[8] have several advantages (e.g. ease of production, cheap, simple purification, high stability compared to enzymes derived from animals or plants). Therefore, they have found many applications in the food and industrial fields. Furthermore, the microorganisms containing lipases are able to perform on broader substrates, which gives more flexibility in various fields. Yeast lipases are frequently secreted out of the cell and are therefore easy to harvest and purify. This opens up their use in a lot of areas. These properties make lipases crucial in the field of biotechnology playing a pivotal role in the sustainable conversion of biological materials into valuable products [9, 10].

Therefore, the present study aimed to isolate yeasts from marine and oil-contaminated environmental samples in Basra, Iraq, identify selected isolates using conventional and *ITS*-based molecular methods, and qualitatively screen their ability to produce extracellular lipase using Spirit Blue Agar and modified Rhodamine B olive oil agar. In addition, Sudan Black B staining was used for the qualitative detection of intracellular lipid accumulation in the selected yeast isolates.

III. Materials and Methods

2.1 Sample collection

80 water and soil samples were collected from various locations in Basra. The first location involved soil contaminated with oil, followed by decomposed agricultural soil, then oil-contaminated soil, followed by pond and swamp water, and finally seawater. Approximately 300 grams of the different soil types were collected from depths of 7 to 15 cm, along with 300 milliliters of water. All necessary data were recorded, and the samples were then transported to the laboratory and stored at 4°C. (Table 1)

Table 1: Sample collection locations.

NO.	Geographical Location		Sample type
1	West Qurna Field 1		Oil-contaminated soil
2	West Qurna Field 2		
3	South Rumaila Field		
4	Chinese drilling tower	HH33	
5		L86	
6		79.5	
7	Automobile oil collection places in Al-Sadiq, Al-Qurna District		Oil-polluted
8	Khor Al Zubair Port		Sea water
9	Great Faw Port		
10	Grass swamp		Pond and swamp water
11	Decomposed agricultural soil, decomposed fruits		Farm soil of Al-Sadiq district

2.2 Isolation and culturing of samples

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Yeasts were isolated according to [11] using the serial dilution technique. One gram of soil, or one milliliter of water from each sample, was transferred to a test tube containing 9 milliliters of sterile Distilled Water (D. W) to prepare the first dilution (10^{-1}) up to the (10^{-5}) dilution. 0.5 milliliters of each dilution were withdrawn using a sterile pipette and spread onto plates containing Sabouraud Dextrose Agar Medium (SDA). The plates were incubated for 48 hours at 30°C to allow the formation of initial yeast colonies.

2.3 Microscopic examination of yeasts

After purifying the colonies growing on SDA medium, microscope slides were prepared by staining them with lactophenol cotton blue. The slides were then examined under a light microscope at 40x and 100x magnification to observe the morphological characteristics of the yeasts, including the shape of the yeast cells and their budding pattern.

2.4 Identification of yeast isolates using the VITEK-2 compact system

According to the manufacturer's protocol for the VITEK-2 Compact system, the yeast suspension was prepared. After purification and activation of the isolates, (1-3) colonies, aged (24-48) hours, were transferred to a sterile tube containing physiological saline. The prepared suspension was placed on a VITEK2 identification card, and the samples were then inserted into the system for automated analysis. Sample identification was performed using the pre-programmed database within the system, according to the procedures described in [12, 13] .

2.5 Molecular study

2.5.1 Genomic DNA extraction

Genomic DNA was extracted from the isolated yeast strains for molecular identification based on the *ITS* gene sequence. The extraction was performed using the Geneaid kit according to the manufacturer's protocol provided with the Presto Mini gDNA Yeast Kit.

Amplification of the *ITS* gene from the genomic DNA was carried out using the polymerase chain reaction (PCR) technique, following the procedure described in reference, with specific forward and reverse primers targeting the gene of interest. (Table 2)

Table 2: Primers

Primer	'3--------'5	Primer length
F-ITS	'5TCCGTAGGTGAACCTGCGG3'	19bp
R-ITS	'5TCCTCCGCTTATTGATATGC'3	20bp

Table 3: Mixture used

Component	Volume
Go Taq Green Master Mix (promega)	25 µl
FWD	2 µl
REV	2 µl
Nuclease-free water	16 µl
gDNA	5 µl
Total	**50 µl**

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The polymerase chain reaction (PCR) was performed using a thermal cycler according to the program outlined in (Table 4).

Table 4: Program PCR

Stage	Step	Temperature and Time	Number of cycles
1	Pre-denaturation	94 °C &3 min	1 cycle
2	Denaturation	94 °C & 40 sec	35 cycles
	Annealing	55 °C &1 min	
	Extension	72 °C &1 min	
3	Final extension	72 °C &10 min	1 cycle

The agarose gel was run at a voltage of 85 V for 50 minutes, and the DNA was visualized within the gel using ultraviolet (UV) illumination.

2.5.2Sequence analysis and phylogenetic tree construction

The phylogenetic tree was constructed using MEGA X software based on aligned *ITS* sequences. Reference sequences were retrieved from the NCBI GenBank database after BLASTn comparison. Multiple sequence alignment was performed before tree construction, and bootstrap analysis was used to evaluate the reliability of clustering. The outgroup sequence was included to root the tree and separate the studied yeast isolates from distant fungal taxa.

2.6 Screening lipase enzyme in yeasts

Spirit blue agar was prepared by dissolving 32.15 g of the medium in 1 liter of D. W, then sterilized using an autoclave. After sterilization and when the medium reached approximately 50°C, olive oil was added as a carbon source and as a lipase enzyme stimulator, and the mixture was thoroughly mixed. It was then poured into Petri dishes and allowed to solidify [14, 15].

2.7 Secondary detection of lipase enzyme activity using modified Rhodamine B olive oil agar medium

This medium was modified to meet the growth requirements of yeasts. It was prepared by dissolving 3 g of PDA powder, 0.2 g of Meat extract, and 0.1 ml of olive oil (as a catalyst for lipase enzyme activity) in 100 ml of D. W. After sterilization, 200 microliters of Rhodamine B solution were added, then poured into Petri dishes and left to solidify. The medium was inoculated with yeast colonies under study, then the plates were incubated in an incubator at a temperature of (30 °C) for (24-72 hours). After incubation, the plates were examined under ultraviolet (UV) light, where the appearance of an orange/fluorescent halo around the colonies indicates lipase enzyme activity as a result of lipid hydrolysis [16]. The absence of fluorescent halos indicates the absence of lipase enzyme.

2.8 Lipid accumulations in yeast cells

According to a previous study [17], yeast was cultured on a nitrogen-limited solid medium consisting of 7 g of potassium phosphate, 2 g of ammonium sulfate, magnesium sulfate heptahydrate, 2 g of sodium monophosphate, 1 g of yeast extract,20 g agar, and 0.05 g of chloramphenicol, dissolved in 1000 ml of D. W, with the pH adjusted to 6.5. After sterilization, 40 g of glucose (as the primary carbon source) was added.

The yeast was inoculated onto this solid medium and incubated for a specified period under appropriate conditions. Following the incubation period, Lipid accumulation in yeasts was monitored using Sudan Black B stain (SBB) as described by [18, 19]. Yeast smears were transferred to a glass slide to prepare a thin cellular smear. The slide was left to dry at room temperature, then heat-fixed by treating the smear with a mixture of absolute

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ethanol and petroleum ether in a 1:1 ratio for 2.5 minutes. After drying, SBB stain (0.3% in 70% alcohol) was added to the slide, and the stain was left on the cells for 15-25 minutes. This stain colors the lipids inside the cells black, distinguishing them from other cellular components. After the staining period, the slide was gently washed with xylene to remove excess stain. A counterstaining step was performed using Safranin, which colors the non-lipid cells, helping to differentiate the lipids from other parts of the cells. The slide was then washed with distilled water and thoroughly dried before being examined under a light microscope at X100 magnification.

IV. Results

3.1 Macroscopic and Microscopic characterization of yeast isolates

The results showed significant variation in cell morphology upon microscopic examination of the purified yeasts. Some isolates exhibited multiple cell shapes, including budding, oval, and spherical forms. Additionally, some cells were rod-shaped or lemon-shaped. Yeast colonies grown on SDA medium also displayed variations in shape, color, texture, and surface luster. (Figure 1)

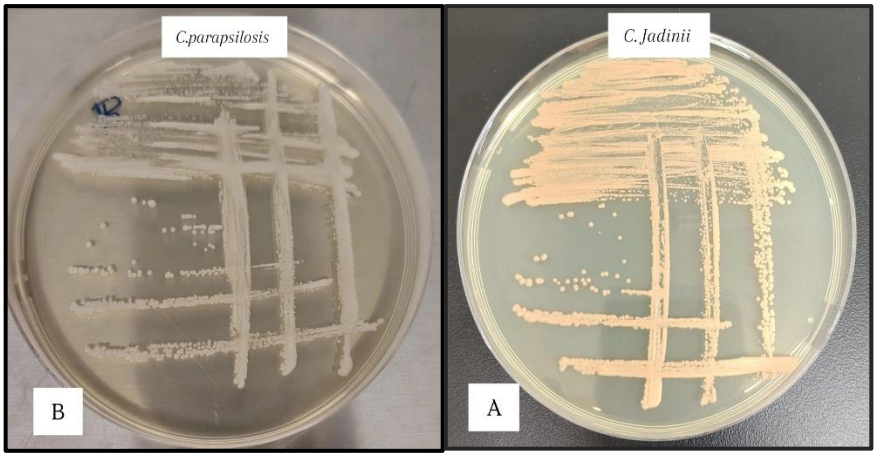

Figure 1: Cultivation of yeasts on SDA medium. A-*Cyberlindnera jadinii*. B-*Candida parapsilosis*

3.2 Identification of yeast isolates using the VITEK2 system

The results, using the VITEK2 system, showed that the yeasts studied included *Candida parapsilosis and Rhodotorula glacialis / mucilaginosa / (Crypto. lauretii)*. (Table5)

Table 5: Diagnosis of yeast isolates using the VITEK-2

Yeast	Bionumber	Probability
Rhodotoruta glutinis/mucilaginusa/ (Crypto.taurenti)	4516105047221511	%94
Candida parapsilasis	4512544265133351	%95
Candida parapsilasis	4102544261111371	91%

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Preliminary identification using the VITEK-2 system suggested that the studied yeast isolates included *Candida parapsilosis* and *Rhodotorula glutinis/mucilaginosa / (Crypto.taurenti)* .Because one isolate showed an ambiguous biochemical identification, *ITS*-based molecular identification was subsequently performed to confirm species identity.

3.3 Molecular study

PCR results showed the presence of DNA strands on agarose gel. (Figure 2)

The *ITS1-ITS4* gene sequences of the three yeasts isolates were compared with the NCBI database. The isolates showed similarity to various genes, including *Cyberlindnera Jadinii* (537 base pairs) with a 100% match, *Candida parapsilosis WISP1* (491 base pairs) with a 100% match and *Candida parapsilosis WISP3* (497 base pairs) with a 99.60% match (Table 5).

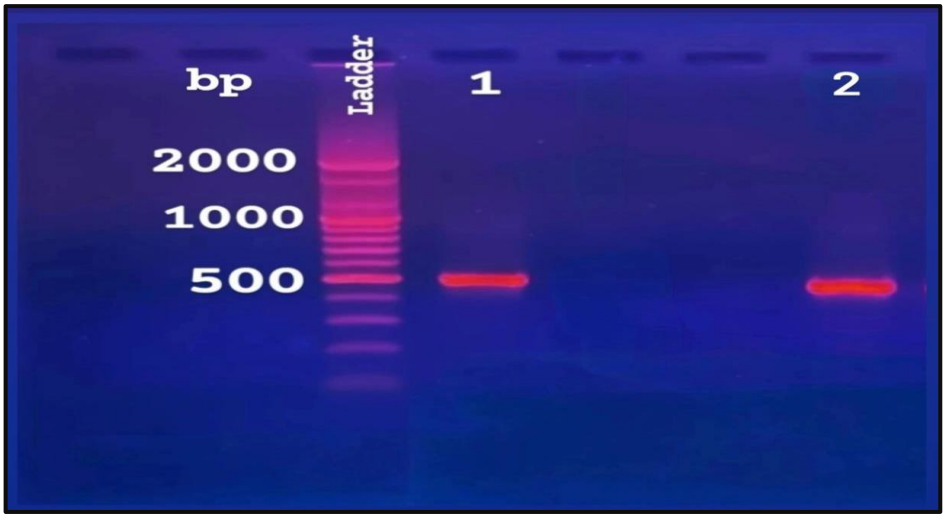

Figure 2: The amplification of the target yeasts using polymerase chain reaction (PCR) products on agarose gel.

Table 6: Percentage of match between the isolated yeasts in the study and the reference samples in the gene bank, and the serial number of each of the reference samples and the yeasts registered in the gene bank.

The samples in this study	Accession No.	GenBank samples	Accession No.	DNA Identity%
Cyberlindnera jadinii	PV602794	*C.jadinii*	KY103057.1	100%
Candida parapsilosis WISP3	PX091468	*C.parapsilosis*	PV416729.1	99.60%
Candida parapsilosis WISP1	PX091466	*C.parapsilosis*	MZ375366.1	100%

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3.4 The phylogenetic tree of yeast isolates

The results of the genetic sequence analysis extracted from the studied yeast isolates using the *ITS* gene (*ITS1–ITS4*) were presented. The evolutionary tree was constructed using the MEGAX program. (Figure3)

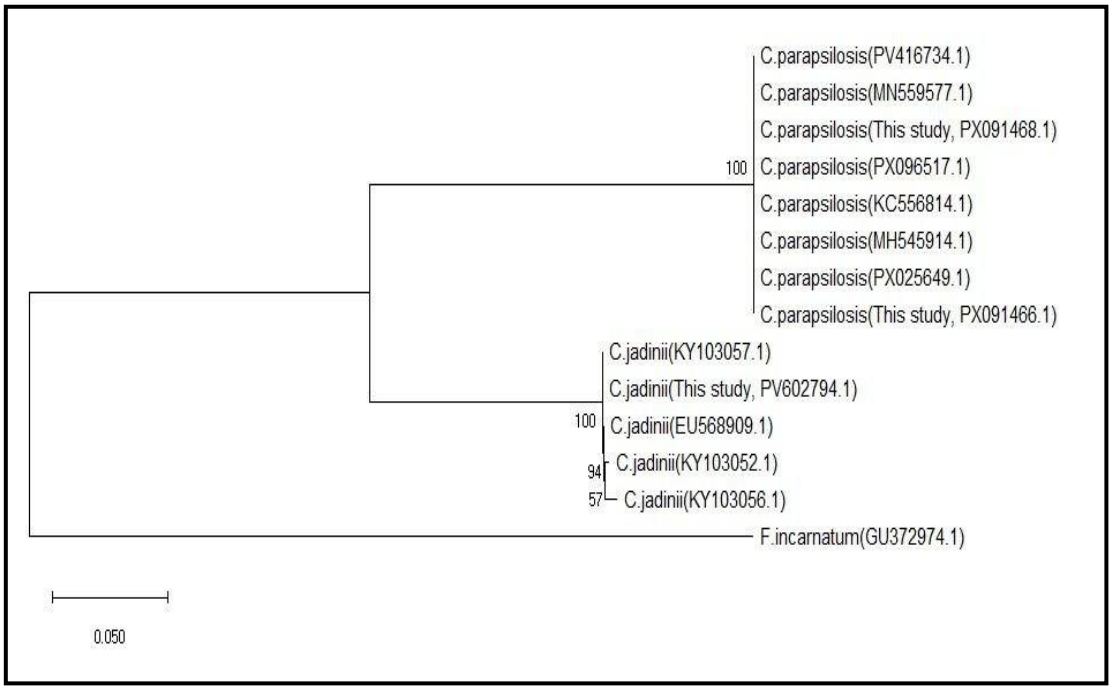

Figure 3: Illustrates the phylogenetic tree of the isolated yeast strains.

The phylogenetic tree showed the clustering of isolates into two main groups representing the species *Candida parapsilosis* and *Cyberlindnera jadinii*. The *Candida parapsilosis* isolates from this study clustered with the reference sequences of the same species in a single clade supported by a bootstrap value of 100%, confirming their identity with this species. The *Cyberlindnera jadinii* isolate was positioned within an independent clade alongside the reference isolates, with bootstrap values ranging between 57–100%. *Fusarium incarnatum* was used as an outgroup, clearly separating from the studied yeast isolates, supporting the validity of the phylogenetic tree construction.

The evolutionary analysis results based on the *ITS* region demonstrated the efficiency of this region in distinguishing between the studied yeast species. The clear clustering of *Candida parapsilosis* isolates with the reference sequences and the high bootstrap value indicates the accuracy of the molecular identification of these isolates. The independent branching of the *Cyberlindnera jadinii* isolate from *Candida parapsilosis* further confirmed the *ITS* region's ability to differentiate between different yeast species. The moderate bootstrap values within the *C. jadinii* clade may reflect limited genetic variation within the species. These results confirm that the use of the *ITS1–ITS4* primers is suitable for molecular diagnosis and studying the evolutionary relationships of yeasts at the species level.

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To provide a clearer overview of the recovered lipase-producing yeasts, the main characteristics of the positive isolates, including sample source, identification method, GenBank accession number, lipase screening result, and lipid accumulation ability, are summarized in (Table 7).

Table 7: Summary of the isolated yeast strains, sources, identification methods, and screening results.

Isolate code	**Sample source**	**Location**	**Preliminary VITEK-2**	**ITS-based identification**	**GenBank accession number**	**Lipase screening result**	**Lipid accumulation**
WISP 1	Oil-Contaminated soil	West Qurna Field 1	*Candida parapsilosis*	*Candida parapsilosis*	PX091466	Positive	Positive
WISP 3	marine sediments	Basra, Khor-AL-Zubair	*Candida parapsilosis*	*Candida parapsilosis*	PX091468	Positive	Positive
Cj isolate	Oil-Contaminated soil	West Qurna Field 1	*Rhodotorula glutinis/mucilaginosa / (Crypto.taurenti)*	*Cyberlindnera jadinii*	PV602794	Positive	Positive

The summarized data show that the three positive isolates belonged to two yeast species. Two isolates were identified as *Candida parapsilosis*, while one isolate was confirmed as *Cyberlindnera jadinii* based on *ITS* sequence analysis. All three isolates showed positive lipase activity in the qualitative screening assays, supporting their selection for further enzymatic and lipid accumulation studies.

3.5 Screening lipase enzyme in yeasts

The preliminary screening for lipase activity was carried out using Spirit Blue Agar (SBA) supplemented with olive oil as a lipid substrate. The results showed that three yeast isolates, representing two yeast species, exhibited positive lipolytic activity: *Cyberlindnera jadinii*, *Candida parapsilosis WISP1*, and *Candida parapsilosis WISP3*.

Positive lipase activity was indicated by the formation of transparent or clear zones around the yeast colonies after incubation for approximately 72 hours at 30°C. In contrast, isolates that did not produce any visible transparent zone or change in the medium were considered negative for detectable lipase activity under the tested conditions (Figure 4).

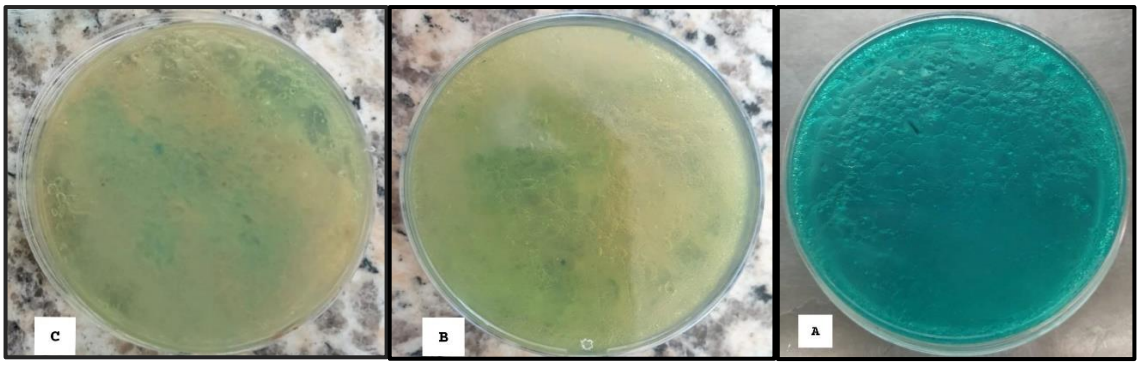

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Figure 4: Growing yeasts on Spirit Blue Agar medium. A-control. Positive samples (B- *Cyberlindnera jadinii* C- *Candida parapsilosis*).

To confirm the preliminary screening results, the three lipase-positive isolates obtained from SBA medium were further cultured on modified Rhodamine B olive oil agar (RBOA) medium. The results confirmed lipase production in *Cyberlindnera jadinii*, *Candida parapsilosis WISP1*, and *Candida parapsilosis WISP3*.

The positive isolates produced bright orange fluorescent halos around the colonies when the plates were exposed to ultraviolet light after incubation. The appearance of these fluorescent halos was considered a positive qualitative indication of lipase activity (Figure 5).

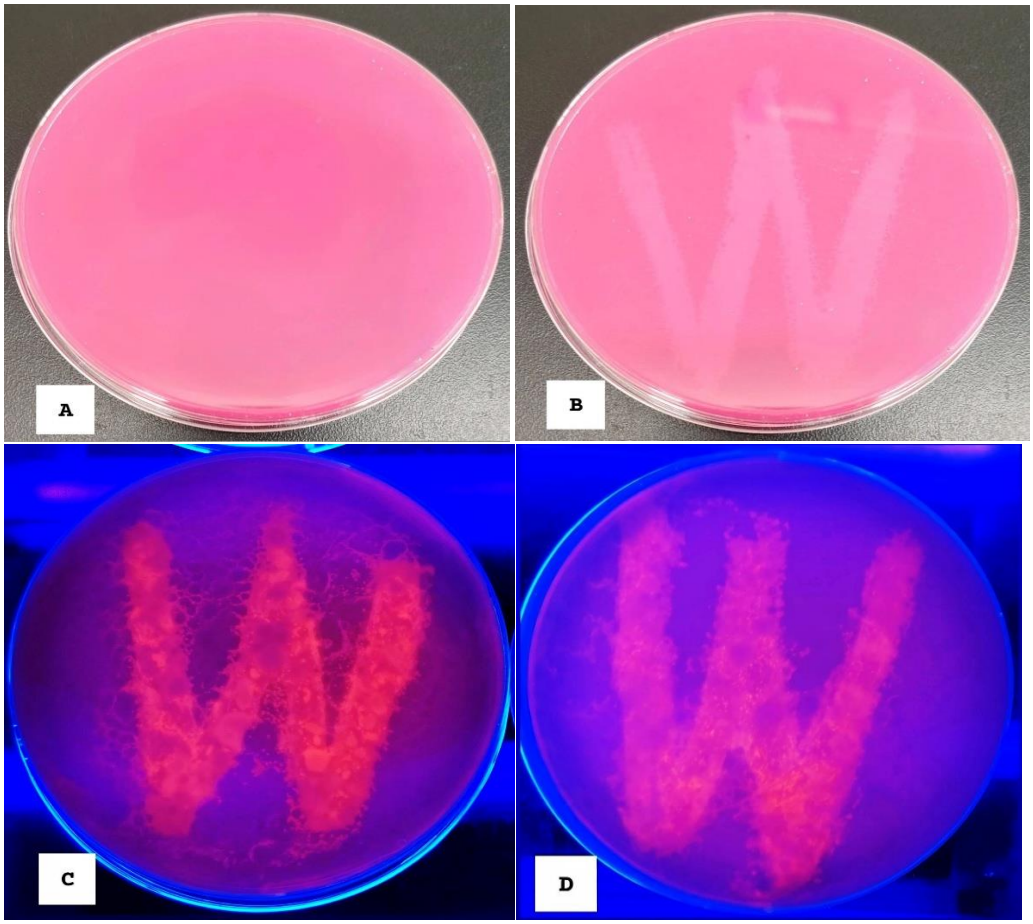

Figure 5: Yeast cultivation on modified Rhodamine B olive oil agar medium to detect lipase enzyme activity. A - Without cultivation.

B - After the required incubation period for the isolated yeast, 24 hours without UV. Appearance of orange fluorescent halos (positive result) in both C - Yeast *C. jadinii*

D - Yeast *C. parapsilosis*

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3.6 Lipid accumulation

The results obtained from the three yeast isolates previously selected and cultivated on solid nitrogen-limited medium showed a positive indication of lipid accumulation when examined using the modified Sudan Black B staining method.

Microscopic examination revealed the presence of lipid bodies inside the yeast cells, appearing as gray to black stained structures, as shown in (Figure 6). The results showed that *Cyberlindnera jadinii* and *Candida parapsilosis* were capable of accumulating lipids within their cells when grown under nitrogen-limited and carbon-rich conditions, with glucose as the carbon source.

The dark gray to black coloration observed inside the cells indicated the accumulation of intracellular lipids. These stained structures represent lipid droplets or lipid bodies that reacted positively with the modified Sudan Black B stain. The distribution of the stain within the cytoplasm further confirmed that lipid accumulation occurred intracellularly. Therefore, these results provide clear qualitative evidence that the tested yeast isolates possess a good ability to accumulate lipids under nitrogen-limited conditions.

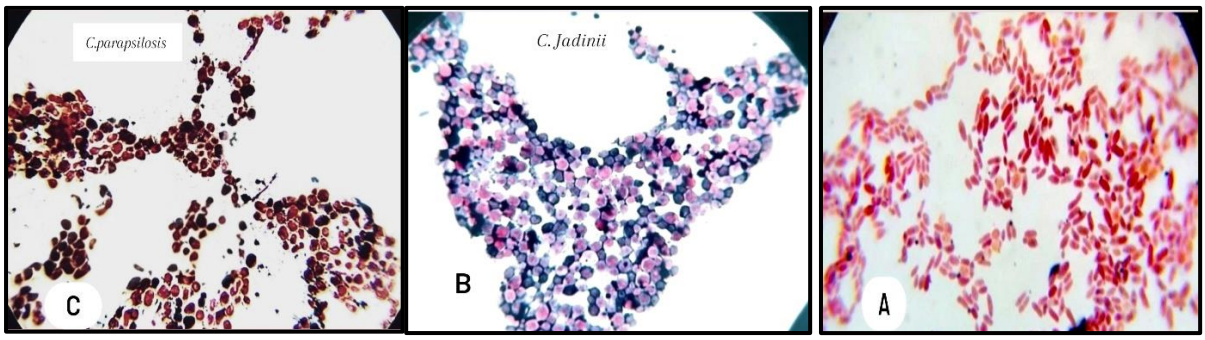

Figure 6: Staining of yeast isolates with Sudan Black B.A stain.

A: No lipid accumulation in the cells of one yeast isolate.

B: Lipid accumulation in the cells of *C. jadinii* yeast.

C: Lipid accumulation in the cells of *C. parapsilosis* yeast.

V. Discussion

In clinical contexts, these enzymes may also be associated with virulence-related processes, including host interaction and modulation of immune responses [20-24]. Therefore, the qualitative detection of lipase activity in the present *C. parapsilosis* isolates agrees with previous evidence that this species can produce extracellular lipolytic enzymes.

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On the other hand, *Cyberlindnera jadinii*, formerly known as *Candida utilis*, is commonly discussed in relation to biotechnological applications [25]. Previous studies have reported lipase production by *Candida utilis* under suitable fermentation conditions [26, 27]. Therefore, the positive lipase activity observed in the present *C. jadinii* isolate indicates its ability to produce extracellular lipase under the applied screening conditions. However, this interpretation remains preliminary because the present study did not quantify enzyme activity or compare lipase production efficiency among isolates.

The results of Sudan Black B staining indicated intracellular lipid accumulation in the tested yeast isolates under nitrogen-limited and carbon-rich conditions. The appearance of dark gray to black intracellular bodies suggests the presence of lipid storage structures within the yeast cells. Sudan Black B staining has been used as a simple qualitative method for detecting lipid accumulation in microorganisms, including yeasts and bacteria [18, 19, 28-30]. However, this method does not provide quantitative information about lipid content, lipid yield, or fatty acid composition.

Overall, the findings of the present study indicate that *Cyberlindnera jadinii* and *Candida parapsilosis* isolates recovered from environmental samples in Basra showed qualitative evidence of extracellular lipase activity and intracellular lipid accumulation. These characteristics may be relevant for future studies related to lipid hydrolysis, microbial enzyme production, and microbial lipid accumulation. However, the current study provides qualitative screening evidence only.

A major limitation of this study is the absence of quantitative measurements for lipase activity and lipid accumulation. The study did not determine enzymatic activity in U/mL, halo diameter, lipolytic index, lipid yield, biomass production, or fatty acid profile. Therefore, the industrial applicability of these isolates cannot be confirmed at this stage. Future studies should include quantitative lipase assays, measurement of halo diameter, calculation of lipolytic index, optimization of culture conditions, enzyme purification, and biochemical characterization of the produced lipase to evaluate the real biotechnological potential of these yeast isolates.

VI. Conclusions and Recommendations

The present study demonstrated that marine and oil-contaminated environments in Basra harbor lipase-producing yeasts. Molecular identification based on *ITS* sequencing confirmed the isolates as *Cyberlindnera jadinii* and *Candida parapsilosis*. Qualitative screening using Spirit Blue Agar and modified Rhodamine B olive oil agar indicated extracellular lipase production, while Sudan Black B staining suggested intracellular lipid accumulation under nitrogen-limited conditions. However, further quantitative enzymatic assays, optimization of culture conditions, lipid extraction, and enzyme characterization are required to evaluate their industrial applicability.

VII. References

.1 Sharma, R., Y. Chisti, and U.C. Banerjee, *Production, purification, characterization, and applications of lipases.* Biotechnology advances, 2001. **19**(8): p. 627-662.

.2 jaffar jabbar Alkabee, H. and M.A. AlHamdani, *Purification and Characterization of Lipase produced by Pseudomonas aeruginosa isolated from some soils of Basrah Governorate.* Al-Kufa University Journal for Biology, 2015. **7**(2): p. 42-49.

.3 Hasan, F., A.A. Shah, and A. Hameed, *Industrial applications of microbial lipases.* Enzyme and Microbial technology, 2006. **39**(2): p. 235-251.

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https://iasj.rdd.edu.iq/journals/journal/issue/20226 *https://doi.org/10.54174/utjagr.v13ii 224.*

.4 Nimkande, V.D. and A. Bafana, *A review on the utility of microbial lipases in wastewater treatment.* Journal of Water Process Engineering, 2022. **46**: p. 102591.

.5 Fjerbaek, L., K.V. Christensen, and B. Norddahl, *A review of the current state of biodiesel production using enzymatic transesterification.* Biotechnology and bioengineering, 2009. **102**(5): p. 1298-1315.

.6 Jaeger, K.-E. and T. Eggert, *Lipases for biotechnology.* Current opinion in biotechnology, 2002. **13** :(4)p. 390-397.

.7 Al-Manhel, A.J., *Application of microbial enzymes in dairy products: A review.* Basrah Journal of Agricultural Sciences, 2018. **31**(1): p. 20-30.

.8 Kadhim, K.F., et al., *Evaluation and Characterization of Lipase Production in Aspergillus niger Isolate Qurna for Biotechnological Applications.* Journal homepage: http://iieta. org/journals/ijdne, 2023. **18**(5): p. 1229-1234.

.9 Ali, S., et al., *The recent advances in the utility of microbial lipases: A review.* Microorganisms, 2023. **11**(2): p. 510.

.10 Kadhim, K. and I. Alrubayae, *Study of lipase production and lipids accumulation of oleaginous fungi isolated from oil-rich soil in Basrah.* Scientific Journal of Medical Research, 2019. **3**(12): p. 123-127.

.11 Mulamattathil, S.G., et al., *Isolation of environmental bacteria from surface and drinking water in Mafikeng, South Africa, and characterization using their antibiotic resistance profiles.* Journal of pathogens, 2014. **2014**(1): p. 371208.

.12 Karagöz, A., S. Acar, and H. Körkoca, *Characterization of Klebsiella isolates by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and determination of antimicrobial resistance with VITEK 2 advanced expert system (AES).* Turkish journal of medical sciences, 2015. **45** :(6)p. 1335-1344.

.13 Sanguinetti, M., B. Posteraro, and C. Lass-Flörl, *Antifungal drug resistance among Candida species: mechanisms and clinical impact.* Mycoses, 2015. **58**: p. 2-13.

.14 Starr, M.P., *Spirit blue agar: a medium for the detection of lipolytic microorganisms.* Science, 1941. **93**(2414): p. 333-334.

.15 Abd Alsada, W.B. and I. jmia Abas, *Molecular Profiling of Lipase-Producing Bacillus spp. from Basrah Extreme Soils.* Indonesian Journal on Health Science and Medicine, 2026. **3**(1): p. 10.21070/ijhsm. v3i1. 386-10.21070/ijhsm. v3i1. 386.

.16 Duza, M.B. and S. Mastan, *Optimization of lipase production from Bacillus thuringiensis (TS11BP), Achromobacter xylosoxidans J2 (TS2MCN)-isolated from soil sediments near oilseed farm.* IOSR J. Pharm. Biol. Sci, 2014 :(2)9 .p. 66-76.

.17 Neema, P. and A. Kumari, *Isolation of lipid producing yeast and fungi from secondary sewage sludge and soil.* 2013.

.18 Burdon, K.L., *Fatty material in bacteria and fungi revealed by staining dried, fixed slide preparations.* Journal of bacteriology, 1946. **52**(6): p. 665-678.

.19 Jape, A., A. Harsulkar, and V. Sapre, *Modified Sudan Black B staining method for rapid screening of oleaginous marine yeasts.* International journal of current microbiology and applied sciences, 2014. **3**(9): p. 4.46-1

.20 Toth, R., et al., *Candida parapsilosis secreted lipase as an important virulence factor.* Current Protein and Peptide Science, 2017. **18**(10): p. 1043-1049.

.21 Branco, J., I.M. Miranda, and A.G. Rodrigues, *Candida parapsilosis virulence and antifungal resistance mechanisms: a comprehensive review of key determinants.* Journal of Fungi, 2023. **9**(1): p. 80.

.22 Tóth, R., et al., *Candida parapsilosis: from genes to the bedside.* Clinical microbiology reviews, 2019. **32**(2): p. 10.1128/cmr. 00111-18.

.23 Gácser, A., et al., *Targeted gene deletion in Candida parapsilosis demonstrates the role of secreted lipase in virulence.* The Journal of clinical investigation, 2007. **117**(10): p. 3049-3058.

.24 Tóth, A., et al., *Secreted Candida parapsilosis lipase modulates the immune response of primary human macrophages.* Virulence, 2014. **5**(4): p. 555-562.

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https://iasj.rdd.edu.iq/journals/journal/issue/20226 *https://doi.org/10.54174/utjagr.v13ii 224.*

.25 Sousa-Silva, M., et al., *Expanding the knowledge on the skillful yeast Cyberlindnera jadinii.* Journal of Fungi, 2021. **7**(1): p. 36.

.26 Grbavčić, S., et al., *Effect of fermentation conditions on lipase production by Candida utilis.* Journal of the Serbian Chemical Society, 2007. **72**(8-9): p. 757-765.

.27 Rasool, S., H. Mukhtar, and I. Ul Haq, *Production of an extracellular lipase by Candida utilis NRRL-Y-900 using agro-industrial by-products.* Turkish Journal of Biochemistry/Turk Biyokimya Dergisi, 2014. **39**.(2)

.28 Hartman, T., *The use of Sudan Black B as a bacterial fat stain.* Stain technology, 1940. **15**(1): p. 23-28.

.29 Patel, R., et al., *Sudan dyes as lipid soluble aryl-azo naphthols for microbial staining.* European Journal of Pharmaceutical and Medical Research, 2015. **2**(3): p. 417-419.

.30 Thancharoen, K., et al., *Selection of oleaginous yeasts with lipid accumulation by the measurement of Sudan black B for benefits of biodiesel.* J Pharm Med Biol Sci, 2017. **6**(2): p. 53-37.

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