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Evaluation of Biodiesel Productivity from *Candida parapsilosis* Isolated from Marine Sediments of Khor Al-Zubair in Basra, Iraq

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Abstract

Introduction: The increasing environmental impact of fossil fuel consumption has intensified the search for sustainable biofuel alternatives. Oleaginous yeasts are promising microbial sources for biodiesel production due to their ability to accumulate intracellular lipids. This study evaluated lipid production and biodiesel potential of *Candida parapsilosis* isolated from marine sediments of Khor Al-Zubair, Basrah, Iraq.

Materials and Methods: Seawater samples were collected from Al-Faw Port, Khor Al-Zubair Port, and Shatt Al-Arab (Arvandrud in Persia). Yeast isolates were identified morphologically and molecularly using ITS1–ITS4 primers, with 99.60% sequence similarity to *C. parapsilosis* (NCBI accession PX091468). Lipid accumulation was induced under nitrogen-limited conditions. Dried biomass was quantified, lipids were extracted using Soxhlet with hexane, and fatty acid methyl esters (FAMEs) were produced by acid-catalyzed esterification. FAME composition was analyzed by GC-MS, and the cetane number (CN) was calculated.

Results: The isolate produced 2.260 g/L of dried biomass and 0.6740 g of extracted lipid, corresponding to 29.81% lipid content. Total FAME yield reached 93.11%. Unsaturated fatty acids accounted for 69.48%, with oleic acid (C18:1) predominating at 61.79%, whereas saturated fatty acids constituted 23.63%. The calculated cetane number was 61.29.

Conclusions: *Candida parapsilosis* demonstrated substantial lipid accumulation and high FAME conversion efficiency, producing biodiesel-compatible fatty acid profiles with a favorable cetane number. These findings support its potential as a sustainable microbial source for biodiesel production.

Keywords: Biodiesel, *Candida parapsilosis*, Cetane Number, Fatty Acid Methyl Esters, Gas Chromatography-Mass Spectrometry, Lipid

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Introduction

Emissions of harmful gases, oxides and particulate matter due to burning of fossil fuels have caused the problem of environmental pollution to become even worse.[1] Therefore, it is very important to search for more sustainable and cleaner alternatives that can replace fossil fuels without harming the environment.[2-4] Among these alternatives, one is of particular importance, namely biodiesel. It is a sustainable and renewable fuel which is defined by its biodegradability and lower carbon emissions than conventional diesel[5] making it an important solution in the framework of a low carbon economy. Biofuels have experienced amazing growth for decades and can be categorized in 4 generations (First: Food crops, Second: Non-food crops, Third: Algae, Fourth: Genetically modified organisms / Advanced biofuels).[6] The use of microbial biomass, derived from yeasts or bacteria, is suitable for conversion into biodiesel due to its ability to accumulate high levels of lipids, particularly triglycerides (TAGs). Microbial oils derived from oleaginous yeasts represent a promising alternative for biodiesel production. These oils are mostly found in the form of TAGs (triacylglycerols). The reason why yeast is such a good option for microbial lipid production is because they can store massive amounts of lipids efficiently. Some yeast species, such as *Yarrowia*, *Rhodotorula*, *Candida*, and *Trichosporon*, can store up to 80% of their cell mass under suitable conditions.[7-9] A major advantage of using yeasts in biodiesel production is their rapid growth and good environmental adaptability, making them less susceptible to seasonal and climatic variations compared to other microorganisms. Yeasts also offer greater economic flexibility, as they can utilize diverse carbon sources such as glucose, sucrose, and glycerol. Although the fat content of some yeasts may vary from one species to another, many of these species can maintain stable production, promoting sustainable production in fluctuating environmental conditions. Therefore, oilseed yeasts are an excellent choice for biodiesel production.[10,11] In general, the increasing environmental impact of fossil fuel consumption

has intensified the search for sustainable biofuel alternatives. Oleaginous yeasts are promising microbial sources for biodiesel production due to their ability to accumulate intracellular lipids. This study evaluated lipid production and biodiesel potential of *Candida parapsilosis* isolated from marine sediments of Khor Al-Zubair, Basrah, Iraq.

Materials and Methods

Sample Collection

The study samples were obtained from seawater (Al-Faw Port, Khor Al-Zubair Port, and Shatt Al-Arab) in Basra Governorate, southern Iraq. 300 ml of each sample of water were collected, stored in sterile, sealed plastic containers, and transported to the laboratory.

Initial Isolation of Yeasts

Samples were serially diluted according to a specific method by Mulamattathil et al.[12] They were cultured on Sabouraud Dextrose Agar (Sigma, USA) and purified to obtain pure colonies. Subsequently, they were stained with lactophenol blue and underwent biochemical testing.

DNA Extraction

For molecular diagnosis, DNA was extracted according to the instructions in Presto™ Mini GDNA Yeast Kit (Geneaid, Taiwan). The presence of DNA in yeast isolates was detected by NanoDrop (ThermoFisher, USA).

Polymerase Chain Reaction (PCR)

PCR was performed using the method described in by Cl et al.[13] employing specialized primers to amplify the Internal Transcribed Spacer (*ITS*), a genetic region commonly used in yeast identification. The *ITS1-ITS4* primers were used to amplify DNA regions extracted from yeast isolates.[14] For the PCR, each reaction was contained 50 µl of mixture solution consisting of 25 µl of Go Taq Green Master Mix (Promega, USA), 2 µl of each primer, 16 µl of sterilized deionized water, and 5 µl of GDNA. The cycling conditions for PCR were as follows: one cycle of 94 °C for 3 min as the holding time and then 35 cycles of 94 °C for 40 s (denaturation), 55 °C for 1 min (annealing), and 72 °C for 1 min (extension) for the amplification. One cycle of 72 °C for 10 min as final extension. The PCR products were loaded into stained agarose gel pits with 1–3 µl of ethidium bromide dye, and electrophoresis was performed for 50 minutes at 85 V. The separated DNA within the agarose gel was visualized using a UV transilluminator.

Lipid Accumulation and Dry Weight Measurement in Yeast Cells

To obtain the highest possible biomass, yeast was cultured on a nitrogen-limited medium, which consists of 7 g of potassium phosphate, 2 g of ammonium sulfate, 1.5 g of magnesium sulfate heptahydrate, 2 g of sodium monophosphate, 1 g of yeast extract, 0.05 g of chloramphenicol, dissolved in 1000 ml of distilled water, and the pH was adjusted to 6.5. After sterilization, 40 g of glucose (as the primary carbon source) was added according to a study by Neema et al.[15] The process involved taking an appropriate amount of purified and active yeast and incubating it in a 500 ml conical flask containing 250 ml of medium. The incubated flask was then placed in a vibrating incubator for 9 days at 30 °C and 120 rpm. After the incubation period, the biomass was centrifuged at 4000 rpm, the supernatant was discarded, and the sediment that formed at the bottom of the tube was collected. It was then washed three times with sterile deionized water. Finally, the dry weight of the yeast biomass was determined and estimated as described by Li et al.[16] Sudan Black B (Sigma, USA) dye was also used for the visualization of lipids in the yeast

Extraction of Lipids from Dried Biomass of C. parapsilosis Yeast

The biomass harvested from the yeasts was lyophilized using a freeze dryer. Total lipid was extracted from the dried biomass using a sexhlet extractor.[17] The lipid was extracted using 150 ml of hexane at boiling point for 6 hours. The solvent containing the lipid was then collected in sterile glass containers. The hexane was evaporated, the remaining lipid was weighed accurately, and the percentage of lipid relative to the dry mass of the sample was calculated.

Esterification Process

According to a method by Shin et al,[18] the esterification process was carried out to obtain methyl esters of fatty acids. The esterification was performed by adding 100 ml of methanol and 2.5% concentrated sulfuric acid to the oil extracted from the yeast. The reaction continued for 45 min in a water bath at a temperature of 90 °C. After that, 2 ml of hexane was added, then the sample was subjected to centrifugation, and the upper hexane layer was separated using a pipette and placed in a new tube for further analysis.

Microbial Biodiesel Properties

FTIR Analysis

After performing transesterification of the isolated yeasts, the hexane layer was taken and examined using an FTIR device to identify the active functional groups of the FAMEs produced from the isolated yeasts, which are involved in biodiesel production. The examination was conducted within the spectral range of 450-4500 cm^{-1}. The analysis was carried out at the Central Laboratory of the University of Basrah.

GC-MS Analysis

The analysis was performed to obtain the total fatty acid methyl esters (FAMEs) content of yeast-extracted lipids (Table 1).

Table 1. Specifications of the GC-MS Used in the Analysis

No.	GC model	Characteristics	M Spectrometer modle	Characteristics
1	Column Oven Temp	initial 40 °C hold 5 mint. Rate1 10 c/min Final tem 310 to end run.	Ion Source Temp	250 °C
2	Purage Flow	3 ml/min	Start m/z	30
3	Injection Mode	Split Ratio 75:1	Solvent Cut Time	4.00 min
4	Pressure	70699 psi	End Time	40-35.00 min
5	Injection Temp	260 °C	Interface Temp	MSD Transferred line 280 °C
6	Flow Control Mode	Constant Flow	Start Time	4.00 min.
7	Column Flow	1 ml/min	Scan Speed	(1562 N2)
8			Column type	HP-5MS 5% phenyl methyl siloxen type
9			Column type	HP-5MS 5%. phenyl methyl siloxane 30m×250 Um×0.25 mm
10	Total Flow	79 ml/min	ACQ Mode	Scan
11	Split Ratio	30.0	End m/z	600

Calculating the Cetane Number (CN)

The equation mentioned by Nomgboye et al.[19] was used to calculate the cetane number based on the FAME ratios reported in the GC-MS analysis.

Results

Yeast Isolation

The results showed that most of the isolates were of the *Candida* genus, with *Candida parapsilosis* exhibiting the highest frequency among the studied yeast isolates. On culture plates, they appeared smooth and shiny. When stained with lactophenol cotton blue, they appeared elongated spherical to oval in shape (Figure 1).

Figure 1. Phenotypic Appearance of *C. parapsilosis* on MEA.

Molecular Diagnosis

After electrophoresis of the PCR results of the studied yeast isolate, the presence of amplified DNA strands was revealed (Figure 2). The amplification products were analyzed using Sanger sequencing and compared using the BLAST software available on the NCBI website. The yeast isolate showed similarity to *Candida parapsilosis* (a 497 bp) with a 99.60% match (Accession No. PV416729.1).

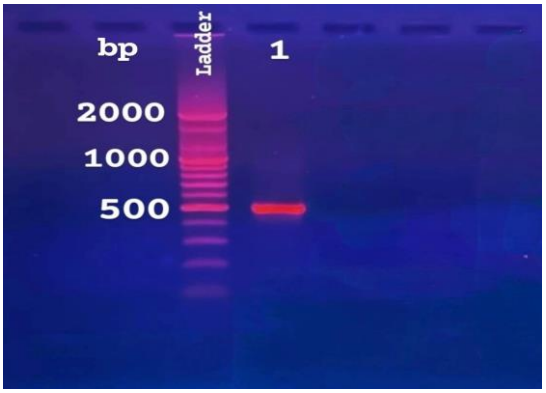

Figure 2. Depictions of the Results after PCR Amplification on an Agarose Gel of the *C. parapsilosis*.

Lipid Accumulation in Yeast Cells

The *C. parapsilosis* yeast isolate in the current study showed a positive result for lipid accumulation when stained with Sudan Black B. Some cells appeared gray to black under the microscope, indicating that the isolate is lipid-rich (Figure 3).

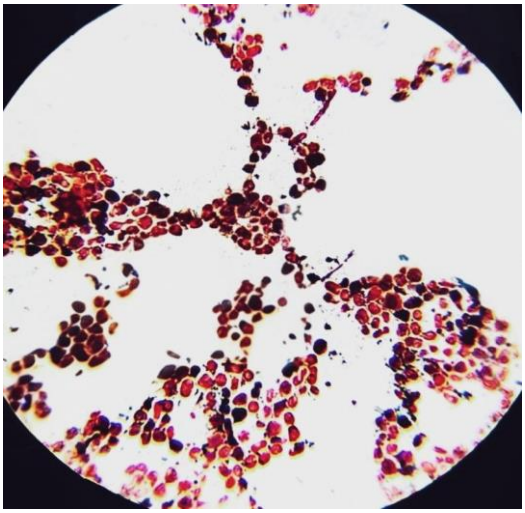

Figure 3. Lipid Accumulation of *C. parapsilosis* Stained with Sudan Black B.

Production of Dried Biomass and Extraction of Total Lipid

After culturing yeast on a liquid nitrogen-limited medium, 2.260 g/L of dried biomass was produced, while the weight of extracted lipid was 0.6740 g. This indicates a total lipid percentage of 29.81% (Figure 4). The percentage of total lipid was calculated according to the following equation:

Lipid percentage = (lipid weight / dry cell weight) × 100.

Figure 4. Dried Biomass of *C. parapsilosis* (A) and Yeast Lipids Extracted with Hexane as a Solvent (B).

Microbial Biodiesel Properties

FTIR Analysis

FTIR spectra of biodiesel produced from *C. parapsilosis* showed the presence of active compounds indicative of biodiesel formation in Figure 5 and Table 2.

GC-MS Analysis

The results after esterification showed that *C. parapsilosis* had a high content of unsaturated fatty acids of 69.48% and a lower content of saturated fatty acids of 23.63%, indicating that the total FAMEs content was 93.11%. Based on the FAME ratio in the GC-MS report, the cetane number of biodiesel produced by *C. parapsilosis* is 61.29 (Figure 6, Table 3).

Table 2. Fatty Acid Methyl Esters Profile of *C. parapsilosis*

Compounds	Active Compounds	Peak(cm^{-1})
Alkane	C-H stretching	2969.84—2830.99
Alkane	C-H bending	1400.07
Esters	C-O stretching	&1180.221099.23

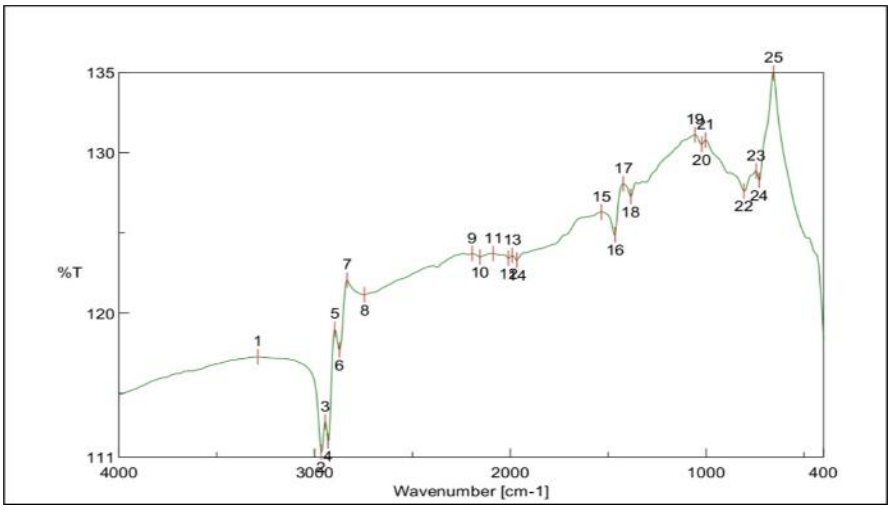

Figure 5. FTIR Plot of *C. parapsilosis*.

Table 3. Shows the Total FAMEs of *C. parapsilosis* as Examined by GC-MS

NC:NDB	Composition of FAMEs %
C16:0 (Palmitic Acid)	17.61
C16:1 (Palmitoleic Acid)	5.16
C17:1 (Margaroleic Acid)	2.53
C17:0 (Margaric Acid)	0.89
C18:1 (Oleic Acid)	61.79
C18:0 (Stearic Acid)	5.13
Total	93.11 %

(NC = Number of Carbons, NDB = Number of Double Bonds)

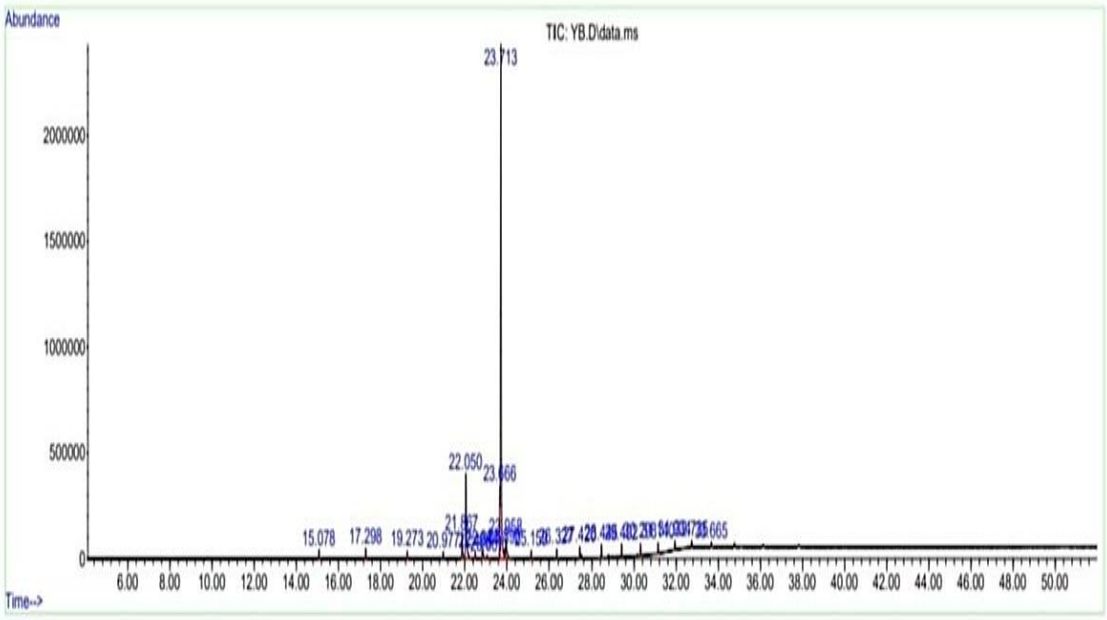

Figure 6. Mass Spectrum of FAMEs in the Biodiesel Analysis Extracted from *C. parapsilosis*.

Discussion

It was noted by Benmessaoud et al.[20] AL-abidy et al.[21] AL-Mosawi et al.,[22] and Aldossary et al.[23] that *C. parapsilosis* is an opportunistic yeast found in various environments. and it is believed to have developed adaptations to survive in harsh environments. Culture results on media are consistent with Gómez-Molero et al.[24]

The accumulation of lipids in yeast cells indicates their ability to accumulate lipids. This result is consistent with previous studies indicating that Sudan Black B staining is used for the preliminary detection of lipid accumulation. This method can be used to detect lipid accumulation, particularly in research on biodiesel production from microorganisms, as it provides preliminary visual indicators without the additional costs associated with using Nile Red staining. This staining can be used prior to lipid extraction by Soxhlet apparatus or GC-MS analysis.

The yeasts used in this study are oleaginous yeast, capable of storing more than 20% of their dry weight in lipid. Specifically, *C. parapsilosis* was stimulated to accumulate significant amounts of lipid, to the extent of 29.81%. Several studies confirm that when yeasts are grown in a nitrogen-limited, carbon-rich environment, they can store lipid in their cells. Instead of forming cellular protein mass, the cells take excess carbon and form fatty acids and form TAGs as an energy store.

The appearance of distinct peaks at (2830.99–2969.84) and 1400.07 indicates C-H stretching vibration, attributed to alkane, which suggests the presence of unsaturated fatty acids. The peaks at 1099.23 and 1180.22 indicate successful esterification and biodiesel formation, attributed to the C-O stretching reactive compounds present in esters. Many studies have shown similar results from these peaks, including Bharti et al.[25] Haq et al.,[26] and Vasaki et al.[27]

C. parapsilosis yeast exhibits lipid-forming capabilities. This observation aligns with what a study by Bukkarapu et al[28] has shown regarding the role of FTIR analysis as a tool for evaluating the composition and quality of biodiesel. The conversion efficiency depends on the type of enzyme or organism used, the nature of the feedstock, and the reaction conditions, as explained by Agu et al.[29]

The fatty acids produced by yeasts are considered one of the main and important factors in determining the properties of biodiesel. The unsaturated fatty acid oleic acid (C18:1) is the most abundant fatty acid in this *C. parapsilosis*, comprising 61.79%. Several studies indicate that oleic acid-rich biodiesel is ideal because it provides suitable viscosity, better combustion, good thermal and oxidative stability, and

a relatively low freezing point. Which makes it desirable in industrial applications. This aligns with the findings of Knothe et al.[30]

Compared to the most oil-producing yeasts, which have a fat content ranging from (40-70%) such as *Yarrowia lipolytica* and *Rhodotorula toruloides*, the conversion rate of oils produced by them to FAMEs ranges between (90-95%). Additionally, the oleic acid in the current study isolated from *C. parapsilosis* is present in similar proportions to that in *Y. lipolytica* and *R. toruloides*, which enhances the quality of the biodiesel produced from these yeasts of Rakicka et al.[31] and Almuhayawi et al.[32]

In the marine isolate of *C. parapsilosis*, its lipid content can be explained as 29.81% of the dry cell weight based on regulatory metabolic response rather than a random trait. When the carbon-to-nitrogen ratio is high and nitrogen limitation occurs, cell division stops, and carbon flow continues through glycolytic pathways and Acetyl-CoA formation, which is redirected toward fatty acid synthesis and triacylglycerol (TAG) accumulation within cellular lipid bodies. This phenomenon has been clearly described in oleaginous yeasts capable of storing more than 20% of their dry weight as lipids under nitrogen-deficient conditions according to Elazzazy et al.[33] and Yang et al.[34] Additionally, harvesting this yeast at the stationary phase enhances accumulation due to the metabolic shift from growth to storage by Beopoulos et al.[35] Furthermore, yeasts often show dominance of oleic acid (C18:1) within the lipid profile under carbon storage conditions, due to its role in maintaining membrane fluidity and physiological balance under stress conditions. Reviews of marine yeasts indicate that species isolated from aquatic environments, including some *Candida* species, exhibit this pattern of lipid accumulation when suitable nutritional conditions are available, supporting the explanation of the recorded percentage according to Kutty et al.[36] and Trofa et al.[37]

The biodiesel produced from this yeast is expected to have higher flammability than conventional diesel, based on the chemical composition of the FAMEs and the calculated cetane number (CN), which reached 61.29. These values are higher than those used in the study by Ahmed et al.[38] where the CN ranged from 48.5 to 55. According to the study, increasing the cetane number reduces ignition delay and improves combustion regularity. Therefore, theoretically, the ignition efficiency of the fuel produced in this study is expected to be high; however, future operational tests must be conducted to assess its actual impact on engine performance. Knowing that the international biodiesel standards are (ASTM D6751 standard in the United States requires a minimum of 47; and the EN 14214 standard in Europe requires a minimum of 51).

Conclusion

This study showed that isolated *C. parapsilosis* is capable of accumulating considerable amounts of intracellular lipids and can be classified as an oleaginous yeast with potential applications in biodiesel production. Analysis of the fatty acids converted to FAMEs indicated that the resulting oils are composed of fatty acids with carbon chains that are suitable for biodiesel production and have a composition similar to that of traditionally used biodiesel oils. Furthermore, the results showed that the biodiesel produced from yeast lipids possesses unique properties, enhancing its potential as an alternative biofuel. The findings support the idea that microorganisms, particularly the *C. parapsilosis*, used in this study, could be a promising microbial source for oil production, thus enabling its use in biodiesel production as a sustainable alternative to fossil diesel.

However, further research is needed to enhance lipids accumulation in different yeast species by controlling growth conditions and the nitrogen-to-carbon ratio. The impact of different carbon sources on the quality and quantity of produced should also be investigated. An evaluation of the biodiesel's physical and chemical properties, compared to established international standards, is essential. More research is crucial to understand the metabolic pathways or genes responsible for lipid accumulation in *C. parapsilosis*, with the aim of improving its productivity.

Authors' Contributions

Authors contributed equally to this study.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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