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Isolation, screening and identification of new lipase producing fungi Talaromyces miniolteus PCK-01-1124 from palm kernel cake in Algeria

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Abstract

Fungal lipases have gained considerable attention due to their broad range of biotechnological applications in industries such as food processing, pharmaceuticals, detergents, biodiesel production, and environmental bioremediation. The increasing industrial demand for these enzymes has stimulated the search for novel lipolytic microorganisms capable of producing efficient and stable lipases. In this context, agro-industrial residues represent promising ecological niches for the isolation of lipase-producing fungi.

In the present study, palm kernel cake (PKC) and crushed date pits (CDP), two agro-industrial by-products rich in residual lipids, were investigated as potential sources of lipolytic fungi. A total of 78 fungal isolates were obtained. Preliminary screening for lipase production was carried out using phenol red agar and rhodamine B agar plate assays. Lipolytic activity was further quantified using a spectrophotometric assay based on the hydrolysis of p-nitrophenyl palmitate (pNPP). Among the isolates, one strain exhibited the highest lipase activity and was selected for molecular identification. Sequencing of the 18S rRNA gene followed by phylogenetic analysis revealed that the isolate belongs to *Talaromyces minioluteus* (PCK-01-1124, PV670919.1).

These findings demonstrate that PKC represents a valuable reservoir of lipase-producing fungi and highlight its potential for the discovery of novel microbial sources of industrially relevant enzymes.

Introduction

Lipases (triacyl-glycerol acylhydrolases, EC 3.1.1.3) are hydrolases involved in the hydrolysis of long-chain acylglycerols in aqueous systems, and in the conditioning of several synthesis reactions in organic media, at the organic-aqueous interface (Chandra et al., 2020; Sood, et al. 2023; Riyadi et al., 2024). Lipases have become the largest group of the commercial enzyme market in the world today due to due to its several characteristics such as the ability to catalyze multifarious actions, specificity, and selectivity, rendering it as one of the precious groups of enzymes, and their market is projected more than USD 797.7 million until 2025 with a compound annual growth rate of 6.2% from 2017 to 2025 and its marketing profile will ride with a compound annual growth rate of 6.4% from 2023 to 2050 (Fatima et al., 2021;Riyadi et al., 2024; Kumar et al., 2024;Abdelaziz et al., 2025).

Lipases have a variety of commercial applications, including in the production of detergents, biodiesel, dairy products, fats and oils, and organic syntheses. They are also used in fat modification, flavour enhancement in food preparation, resolving race-mic mixtures, and in the pharmaceutical industry and the biodegradation of post-consumer polyethylene terephthalate (PET) packages (Moyses et al., 2021;Al-Bedak et al., 2025).

These lipases are widely found throughout secreted from plants, animals, and microorganisms (Adetunji & Olaniran, 2021;Geoffry & Achur, 2018) to extract and purify lipases from fungi is often simpler and more cost-effective than from other sources (Kumar et al., 2023). The fungal lipase is exploited due to its

low cost of extraction, thermal and pH stability, substrate specificity "enantioselectivity", enhanced activity in organic solvents, and genetic manipulation is ease; these characteristics make them highly suitable for industrial applications. It is a specific enzyme that digests fat and is characterized by its ability to hydrolyze fat over a wide range of temperatures and pH. It also regulates the levels of cholesterol and triglycerides and is helpful in different dietary regime for weight management. It is ideal for use in leather, detergent and cosmetic industries (Athukorala & Samaraweera, 2023).

The most producer's species are *Rhizopus sp., Geotrichum sp., Mucor sp., Aspergillus sp., and Penicillium sp.*, within the genus of *Aspergillus sp., A.niger* and *A.oryzae* are on the generally recognized as safe (GRAS) list of the Food and Drug Administration (FDA) in the United States (Prabaningtyas et al., 2018).

Palm kernel cake (PKC) is the solid residue obtained after oil extraction from the kernels of the oil palm (Elaeis guineensis Jacq.) (Tsouko et al., 2017). It is one of the major by-products of the palm oil industry, generated in millions of tons annually worldwide (Mijiyawa et al, 2026) This by-product is generally a good source of fat, protein, minerals and carbohydrates Indeed, its chemical composition shows that it contains approximately 14–18% crude protein, 12–20% crude fiber, 3–9% crude fat, and various minerals (Azizi et al., 2021). Owing to this, particularly its residual lipid content, PKC constitutes a lipid-rich ecological niche that may selectively harbor lipolytic microorganisms, making it a promising for the bioprospection of novel lipase-producing fungi.

The aim of the current research was undertaken to explore the mycobiota of PCK as a source of novel lipolytic fungi, combining robust screening strategies with morphological and molecular tools to characterize the most active isolates and assess their biotechnological potential.

Materials and Methods

Sample collection

08 samples of Palm Kernel Cake (PKC) were collected from a palm oil factory in Bordj Bou Areridj, Algeria in sterile bottles. The samples were transported to the Biotechnology Research Center laboratory at 4°C for analysis and stored at the same temperature (Riyadi et al., 2017).

7 distinct Algerian date kinds (Tentabouche, Tafezoine, Utaqbala, Tadelet, Kenetichi, Sbaa laarous and Degla Beida) arrive from market place in Constantine city (East of Algeria) to use the pits, they were crushed by using mortar and pestle to pound, then RETSCH ZM 200 Model Ultra-Centrifugal Mill used to ring sieves with aperture sizes 1.5 cm.

Microorganism and inoculum preparation

Collected PKC_S were used for analysis of the fungal population using potato dextrose agar (PDA) medium. After autoclaving, a serial dilution approach has been used to isolate the fungal species

(Usman et al., 2023). All isolation plates were incubated for 7 days at temperature of 30°C (Dix, 1995).

Isolates have been purified using single- spore isolation method to obtain a pure culture of the fungus described by Noman et al. (2018).

Microscopic Identification of the fungal isolates

Initially detection of fungal samples, conduction based on visual observation in culture in petri dish and microscopic morphological research in slide culture. Cultures were grown on PDA plate in the center for 7 days at 28°C and their growth rate, color change, zonation and sporulation were observed. The morphology of the isolates was analyzed with microscope and imaging software (Olympus CX21FS1). In the microscopic examination, we used a stain using lactophenol cotton blue (Surajit,et al., 2009).

Strain Conservation

Storage of the fungal strain was by using glycerol-slice prepared from 50% sterile glycerol placed on a fungal surface of 0.5 cm diameter collected from the actively growing areas of the colonies, the method lasts longer than other (Saxena & Gupta, 2019).

Fungi preparation and extracellular lipase production

The fungal strains were screened using agar plate technique and validated later using Rhodamine B plate assays and red phenol plate for the real production of extracellular lipase.

Yeast extract-peptone medium (YP) was media used for growing the fungus, it generated by 1 liter of 0.1 M phosphate buffer, pH 7.0 comprising 10 g peptone and 1 g yeast extract, then autoclaving (Roberts et al., 1987). After, addition of 1% sterilized glucose to the sterile YP solution.

Incubated at 30°C and 110 rpm in a rotary shaker of Sterile Erlenmeyer flasks (250 ml) containing 100 ml sterile yeast-extract-peptone medium with glucose were inoculated with 2 ml (1×10^7 spores/ml) of spore suspension of 7-day-old culture.

After 5 days, the cultures were filtered with Whatman No. 1 filter for separation of mycelium (Riyadi et al., 2017) and then pressured with muslin cloth and centrifuged for 15 min at 5000×g and 4°C (El-Ghonemy et al., 2017). Then, the supernatant was passed sterile filters of 0.22 µm to eliminate any microorganisms, then the resulting supernatant was used for the lipase test.

Sterile culture filtrates were stored at -20°C until use (Roberts et al., 1987) (Gopinath et al., 2005).

Qualitative screening of lipase-producing strains

Qualitative screening method- phenol red agar method

All isolates of fungal strains were determined for lipase activity by the phenol red agar plate method, which was used to assess lipase-producing fungal strains for qualitative screening.

Phenol red agar plates were prepared by Phenol red 0.01%(w/v), $CaCl_2$ 10 mM, olive oil 1% (v/v), and agar 2%(w/v). The pH was adjusted to pH 7.3 ± 2.

In the center of the Petri plates, an actively growing fungal disc of 3 mm was placed. The isolated organisms were inoculated at 28°C for 2 days (El-Ghonemy, 2017) (Riyadi et al., 2017).

A red color shift in to yellow (circular zone) surrounding the colony shows lipase synthesis (enzyme activity) by isolates (El-Ghonemy, 2017)

Qualitative screening method-rhodamine B agar method

Pure strains with fresh colonies have been selected for growth Rhodamine B plates with olive oil as substrate and rhodamine B fluorescent dye (Rabbani et al., 2013). 5 mm diameter plugs of pure and fresh isolates colonies were inserted in the center of the petri plate.

Lipase-producing fungal were identified six days after incubation in plaques at 28°C by the appearance of orange fluorescence halos around the colonies which detected by fluorescence with UV light at 350 nm (Geoffry & Achur, 2018) (Ortellado et al., 2021).

Quantative screening of lipase activity

The method used to quantify lipase was based on quantification of the p-nitrophenolate (p-NP) released by lipolytic enzymes during hydrolysis of p-nitrophenyl acyl esters. The p-NP is chromogenic with a maximum absorption at 410 nm, it was measured by EnSpire Multimode Plate Reader in microplates (20–200 µL reaction mixture).

The modified method described by Jaeger & Kovacic, 2014 using Substrate stock: 20 mM p-NP palmitate in isopropanol.

Yellow color which is the indication of Lipase hydrolyzed p-NPP to p-NP, after 15 min incubation at 37°C one unit (1U) was defined as that amount of enzyme that liberated 1 µmol of p-nitrophenyl palmitate (pNPP) per minute (Gopinath et al., 2005).

Identification of the best lipase-producing isolates

The strain screened as promising for lipase production in terms of hydrolytic activity was identified.

2.8.1. Morphological characteristics by scanning electron microscope (SEM)

For SEM analysis, a fungal mat segment of approximately 0.2 mm comprising spores and mycelia from a 12-day-old colony was prepared in accordance with the protocol (Roy et al., 2018), the colonies were elated on small glass slides (surface area of one cm^2).

Scanning electron microscope (SEM) images of the isolated fungus were obtained at different magnifications to study morphological characteristics (Roy et al., 2018).

Molecular identification

DNA extraction

The strain which showed maximum lipolytic activity was identified by internal transcribed spacer (ITS) sequencing. The genomic DNA was isolated according, the modified method described by Gómez et al., (2024).

Polymerase Chain Reaction Amplification

18SrDNA has been amplified by the polymerase chain reaction (PCR) using the universal primer pair ITS1-F (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4-R (5'- TCCTCCGCTTATTGATATGC-3') in Platinium II Hot Start PCR Master Mix purification kit (Invitrogen, Lituania), following the manufacturer's instructions. Amplifications were conducted under the following thermal cycling conditions: initial denaturation for 3 min at 95°C, followed by 35 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 56°C, and extension for 1 min at 72°C, with a final extension step for 15 min at 72°C (Manova et al., 2022). Amplified products were resolved in a 1.5% agarose gel and detected by ethidium bromide staining. DNA bands were analyzed by UV-based detection method with the Vilber Lourmat Quantum CX5 Edge – Epi UV for TLC. A 100-bp DNA ladder (Quick-Load® 100 bp DNA Ladder, Qiagen, Germany) was used to estimate DNA fragment sizes.

Sequencing and Phylogenetic analyses

The purified DNA obtained from the ITS-PCR was sequenced by the Sanger method (Sanger & Coulson, 1975). The nucleotide sequences were subjected to BLAST analysis on (http://www.ncbi.nih.gov/index.html). Phylogenetic trees have beenconstructed and the fungal strain has been identified by mega 6. The sequences were submitted to NCBI database Gene Bank and accession numbers obtained.

Statistical Analysis

All data collected in this study were analyzed using Microsoft Excel. Descriptive statistics were applied to summarize the total lipase activity. Results are expressed as mean ± standard deviation (SD). These descriptive measures were computed for all strains and conditions examined in the study.

Results

Isolation

Fungi were isolated from two types of agro-industrial substrate samples, palm kernel cake (PCK) and crushed date pits (CDP), using potato dextrose agar (PDA).

From a total of 15 samples, 78 fungal isolates were recovered and classified into six taxonomic groups: *Penicillium sp.*, *Aspergillus sp.*, *Cladosporium sp.*, *Mucor sp.*, *Trichoderma sp.*, and *Alternaria sp.*

Figure 1 Morphological characteristics of *Talaromyces minioluteus* strain PCK-01-1124 (PV670919.1). (a, b) Microscopic examination showing spores at ×100 magnification; (c) obverse colony on PDA; (d) reverse colony on PDA.

Quantitative screening of lipase activity of fungal isolates

The obtained isolates were subsequently screened for their ability to produce extracellular lipase using phenol red agar and rhodamine B agar media.

Among these isolates, 44 exhibited lipolytic activity, corresponding to an overall prevalence of 56.41% (Table 1).

Table 1
The result of the screening study for the lipase-producing fungal isolated from palm kernel cake and crushed date pits.

Source	No. of samples	Strains	No. of total fungal isolates	No. of lipase producing isolates	Percentage	
Palm Kernel Cake (PKC)	08	*Penicillium sp.*	14	09	64.28%	(9/14)
		Aspergillus sp.	32	16	50%	(16/32)
		Cladosporium sp.	01	01	100%	(01/01)
		Mucor sp.	03	02	66.67%	(02/03)
		Trichoderma sp.	02	01	50%	(01/02)
Crushed date pits (CDP)	07	*Penicillium sp.*	08	03	37.5%	(03/08)
		Aspergillus sp.	11	08	72.73%	(08/11)
		Cladosporium sp.	01	01	100%	(01/01)
		Mucor sp.	02	00	0%	(00/02)
		Trichoderma sp.	02	01	50%	(01/02)
		Alternaria sp.	02	02	100%	(02/02)
Total		*Penicillium sp.*	22	12	54.55%	(12/22)
		Aspergillus sp.	42	24	57.14%	(24/42)
		Cladosporium sp.	02	02	100%	(02/02)
		Mucor sp.	05	02	40%	(02/05)
		Trichoderma sp.	04	02	50%	(02/04)
		Alternaria sp.	02	02	100%	(02/02)
	15		78	44	56.41%	(44/78)

As shown in Fig. 2, yellow zones around the colonies as a result of lipolytic activity due to the hydrolysis of the substrate, which results in a color change of phenol red from red to yellow. In Fig. 3, orange fluorescence was observed surrounding the colony under UV light, confirming lipase activity.

Lipase production was generally higher among isolates from PCK, with *Cladosporium sp.* exhibiting 100% activity, followed by *Penicillium sp.* (64.28%), *Mucor sp.* (66.67%), and *Aspergillus sp.* (50%). In contrast,

Trichoderma sp. and *Alternaria sp.* showed moderate lipase production at 50%. Isolates from CDP displayed wider range of lipolytic activity: *Mucor sp.* showed no detectable activity (0%), whereas *Cladosporium sp.* and *Alternaria sp.* reached 100% activity. *Penicillium sp.* and *Aspergillus sp.* showed intermediate activities of 37.5% and 72.73%, respectively.

The forty-four highest lipase-producing isolates were selected from the previous qualitative method, then were screened quantitatively using pNPP. The lipase activity assay of different lipase-producing fungal isolates from two types of samples is expressed in Table 2.

Table 2
Fungal isolates and their corresponding enzyme activity in U/mL

No.	Isolates strain	Source of the fungal isolate	Phenotypic identification	Total lipase activity (U/mL)
1.	**PCK-01-1124**	**Palm kernel cake**	***Penicillium sp.***	**305.87 ± 0.37**
2.	PCK-06-14524	Palm kernel cake	*Aspergillus sp.*	303.02 ± 0.22
3.	CDP-01-1501	Crushed date pits	*Penicillium sp.*	301.77 ± 0.40
4.	CDP – 01-08224	Crushed date pits	*Aspergillus sp.*	231.77 ± *0.84*
5.	PCK-03-15224	Palm kernel cake	*Aspergillus sp.*	164.76 ± *0.40*
6.	PCK-03-15224	Palm kernel cake	*Aspergillus sp.*	163.39 ± *1.15*
7.	PCK-04-6324	Palm kernel cake	*Aspergillus sp.*	116.04 ± *5.05*
8.	PCK-05-1424	Palm kernel cake	*Penicillium sp.*	110.83 ± *0.59*
9.	CDP – 01-08224	Crushed date pits	*Aspergillus sp.*	107.92 ± *0.09*
10.	PCK-O6-14524	Palm kernel cake	*Penicillium sp.*	100.40 ± *0.21*
11.	PCK-05-1424	Palm kernel cake	*Aspergillus sp.*	96.73 ± *0.50*
12.	PCK-05-1223	Palm kernel cake	*Aspergillus sp.*	91.17 ± *1.15*
13.	PCK-06-14524	Palm kernel cake	*Mucor sp.*	83.22 ± *2.08*
14.	CDP – 01-26224	Crushed date pits	*Aspergillus sp.*	78.86 ± *1.61*
15.	PCK-03-15224	Palm kernel cake	*Mucor sp.*	58.95 ± *0.84*
16.	PCK-04-6324	Palm kernel cake	*Penicillium sp.*	58.09 ± *0.40*
17.	CDP – 01-1501	Crushed date pits	*Alternaria sp.*	55.27 ± *0.03*
18.	PCK-01-1124	Palm kernel cake	*Aspergillus sp.*	54.59 ± *0.09*
19.	CDP – 01-08224	Crushed date pits	*Alternaria sp.*	51.94 ± *0.22*
20.	PCK-01-1124	Palm kernel cake	*Penicillium sp.*	50.91 ± *0.03*
21.	CDP – 01-26224	Crushed date pits	*Aspergillus sp.*	49.46 ± *0.09*
22.	CDP – 01-26224	Crushed date pits	*Cladosporium sp.*	49.03 ± *0.03*
23.	CDP – 01-26224	Crushed date pits	*Trichoderma sp.*	44.25 ± *0.22*
24.	PCK-07-16524	Palm kernel cake	*Aspergillus sp.*	43.31 ± *0.09*
25.	CDP – 01-08224	Crushed date pits	*Aspergillus sp.*	41.94 ± *0.06*
26.	CDP – 01-8224	Crushed date pits	*Aspergillus sp.*	28.18 ± *0.09*

No.	Isolates strain	Source of the fungal isolate	Phenotypic identification	Total lipase activity (U/mL)		
1.	**PCK-01-1124**	**Palm kernel cake**	***Penicillium sp.***	**305.87**	**±**	**0.37**
27.	CDP – 01-1501	Crushed date pits	*Penicillium sp.*	27.07	±	*0.22*
28.	PCK-04-6324	Palm kernel cake	*Aspergillus sp.*	25.36	±	*0.00*
29.	PCK-05-21424	Palm kernel cake	*Aspergillus sp.*	19.89	±	*0.40*
30.	PCK-04-6324	Palm kernel cake	*Trichoderma sp.*	18.78	±	*0.40*
31.	CDP – 01-26224	Crushed date pits	*Cladosporium sp.*	15.02	±	*0.03*
32.	CDP – 01-26224	Crushed date pits	*Aspergillus sp.*	14.59	±	*0.09*
33.	CDP – 01-1501	Crushed date pits	*Penicillium sp.*	10.91	±	*0.22*
34.	PCK-07-16524	Palm kernel cake	*Penicillium sp.*	10.32	±	*0.87*
35.	PCK-07-16524	Palm kernel cake	*Aspergillus sp.*	11.05	±	*7.81*
36.	PCK-03-15224	Palm kernel cake	*Penicillium sp.*	7.07	±	*5.42*
37.	PCK-05-21424	Palm kernel cake	*Aspergillus sp.*	6.30	±	*0.12*
38.	CDP – 01-1501	Crushed date pits	*Aspergillus sp.*	5.87	±	*0.37*
39.	PCK-01-1124	Palm kernel cake	*Penicillium sp.*	4.93	±	*0.87*
40.	PCK-03-15224	Palm kernel cake	*Penicillium sp.*	3.82	±	*0.65*
41.	PCK-03-15224	Palm kernel cake	*Aspergillus sp.*	3.82	±	*1.21*
42.	PCK-07-16524	Palm kernel cake	*Aspergillus sp.*	3.05	±	*0.09*
43.	PCK-04-6324	Palm kernel cake	*Aspergillus sp.*	2.45	±	*0.40*
44.	CDP – 01-1501	Crushed date pits	*Aspergillus sp.*	0.74	±	*0.65*

The activity assay results were expressed as means ± SD.

Morphological characteristics by scanning electron microscope (SEM)

SEM micrographs of the isolated fungus at different magnifications are presented in Fig. 4.

Figures 4a to 4d illustrate the overall structure of the fungal mat, which consists of a dense network of septate hyphae that are intricately branched, creating a compact, felt-like texture. The hyphae have a mostly smooth surface, sometimes slightly rough, with some strands weaving together and adhering closely to the underlying surface.

Figures 4b, 4c, and 4d show the shape of the phialides and conidia. The phialides are flask-shaped and elongated, growing either along the sides or at the ends of the hyphae. They have distinct collars at their tips, from which the conidia develop in a sequence that progresses from the base upward. The conidia cluster in sticky, spherical heads at the phialide tips and appear as small, smooth-walled, single-celled structures that range from oval to ellipsoidal in shape.

Figure 4e zooms in on the conidia at very high magnification (100,000x), revealing fine surface details. These conidia are mainly ellipsoidal, about 1 to 2 micrometers in diameter, with a notably thin and smooth outer wall. The micrograph also clearly shows the delicate hilum—the attachment point on some conidia—confirming that they develop blasticly from the phialides.

Molecular identification

The taxonomic position of PCK-01-1124 strain, exhibiting maximum lipolytic activity, using DNA sequence datasets analyzed with MEGA software and the BioEdit Sequence Alignment Editor (Version 7.0.9.0). The aligned datasets were subjected to maximum likelihood (ML) phylogenetic analysis based on the internal transcribed spacer (ITS) region (Fig. 5), which illustrates the phylogenetic relationships between the studied strain and representative species of *Talaromyces*. The ITS sequence of the strain clustered well with strain of *T. minioluteus* OP802636 (bootstrap = 100%, BIpp = 0.99).

Concatenated phylogenetic trees of ITS gene regions were constructed using the ML method, with *T. minioluteus* designated as the ingroup taxon within the *Talaromyces* section Trachyspermi. Bootstrap values obtained from 1,000 replications are shown at the nodes of the tree; bootstrap support values below 50 are not shown.

The strain has been deposited in GenBank under accession number PV670919.1.

PCR amplification of the ITS region for strain PCK-01-1124 yielded a single band of approximately 600 bp (Fig. 6).

Discussion

Microbial lipases have emerged as enzymes of exceptional industrial relevance, primarily due to the inherent advantages associated with microbial production systems, including high volumetric yields, substrate versatility, ease of genetic manipulation, and the capacity for large-scale cultivation on low-cost media (Geoffry & Achur, 2018; Mehta et al., 2020). Among microbial sources, filamentous fungi are particularly valued for their ability to secrete lipases extracellularly at high titers, and their tolerance to a wide range of physicochemical conditions makes them especially attractive for biotechnological applications (Adrio & Demain, 2014).

In the present study, samples were collected from Palm kernel cake (PKC) and crushed date pits (CDP). PKC served as a critical substrate for the isolation of lipase-producing fungi, as previous studies have

highlighted its rich nutritional profile, which promotes microbial growth and enzyme induction. It contains 6% of lipids and significant amounts of crude protein (Iluyemi et al., 2006);(Khairudin et al., 2012), creating favorable conditions for lipase synthesis. These results demonstrate PKC's effectiveness in solid-state fermentation for industrial enzyme production (Horincar et al., 2017) ; (Riyadi et al., 2017).

The presence of lipidic compounds in the substrate is a well-established inducer of fungal lipase biosynthesis, as microorganisms typically upregulate lipolytic enzyme production in response to lipid-rich environments as a carbon acquisition strategy (Treichel et al., 2010; Aloulou et al., 2012). This mechanism explains why agro-industrial residues, such as PKC, have gained increasing attention as both inducers and cost-effective fermentation media for lipase production. Date pits similarly represent a promising lipid-containing substrate, as they have been reported to contain significant amounts of fatty acids and polyphenols that may induce and modulate lipase expression in fungi (Al-Farsi & Lee, 2008).

Lipase production was confirmed using two complementary assays. First, a large-scale screening on phenol red agar identified potential lipase producers by detecting hydrolysis-induced acidification (pH drop), which changed the indicator from orange to yellow (Riyadi et al., 2017). Second, rhodamine B-olive oil agar was used as a highly sensitive method for detecting extracellular lipase activity (Zottig et al., 2016); (Abrunhosa et al., 2013).

Screening for lipase-producing fungi was performed using two complementary and widely validated colorimetric methods. The phenol red-based agar assay, which detects lipase activity through the colorimetric indication of pH-mediated acidification resulting from fatty acid release during triglyceride hydrolysis, provided an effective large-scale primary screening platform (Riyadi et al., 2017; Gopinath et al., 2013). This method is particularly valued for its simplicity, reproducibility, and suitability for high-throughput screening. As a secondary, highly sensitive confirmatory method, rhodamine B-olive oil agar was employed to detect extracellular lipase activity through the formation of fluorescent halos under UV light, attributed to the formation of rhodamine B-fatty acid complexes upon lipolysis (Zottig et al., 2016; Abrunhosa et al., 2013). The combined use of these two orthogonal screening methods reduced the risk of false positives and false negatives inherent to single-assay approaches (Gupta et al., 2015).

Among the 44 fungal isolates screened, strain PCK-01-1124 emerged as the most promising lipase producer, exhibiting a hydrolysis halo diameter of 7 cm on phenol red agar and 4.5 cm on rhodamine B agar yielding an activity index of lipase (I_{AL}) of 0.15. According to Ortellado et al. (2021), indicates a good enzymatic production potential, as a lower I_{AL} value is considered more promising.

To confirm this observation, lipase activity was further evaluated in detail using pNPP as substrate, a technique widely employed for quantitative lipase assays of the different fungal isolates, as done by Alabdalall et al. (2021) and (Jaeger & Kovacic, 2014). Across all 44 isolates, lipase activity ranged broadly from 305.87 ± 0.37 and 0.74 ± 0.65 U/mL, reflecting the considerable diversity in lipolytic potential within the PKC-associated fungal community. According to Table 2, PCK-01-1124 was identified

as a high-potential lipase producer from PKC, consistent with its recognition as a lipase producer (Stošić et al., 2020).

The high-potential lipase producer was identified by 18S rRNA gene sequencing provides a powerful tool for then sequenced, a gold-standard approach for fungal taxonomic placement that offers superior resolution compared to morphological classification alone, particularly within morphologically conserved genera (Samson et al., 2011; Houbraken et al., 2020). In GenBank, the isolate under investigation were tightly clustered with related species. Phylogenetic analysis revealed that the isolate clustered with high bootstrap support within the *Trachysperma* section, sharing close evolutionary affinity with *T. chongqingensis*, *T. minnesotensis*, *T. udagawae*, and most notably *T. minioluteus*. These clustering relationships are consistent with previously published phylogenies of the section (Sun et al., 2020) (Sun et al., 2020); (Stošić et al., 2020), and position the strain as *T. minioluteus* PCK-01-1124 (accession no. PV670919.1, formerly designated *Penicillium* sp.). Phylogenetic analyses of the *Trachysperma* section revealed that *T. chongqingensis* always grouped with *T. minioluteus*, *T. minnesotensis* and *T. udagawae*, with strong statistical support. Figure 5 shows that *T. minioluteus* was the most closely related sister taxon to the isolate, while, another member of the clade, has been explicitly documented as a potent enzyme producer (Sun et al., 2022). Similarly, *T. udagawae* has been reported to exhibit significant lipolytic activity in earlier investigations (Sun et al., 2020); (Stošić et al., 2020), further supporting the notion that lipase production capacity may represent a conserved enzymatic trait across the *Trachysperma* section.

Morphological characterization by scanning electron microscopy (SEM) of *T. minioluteus* PCK-01-1124 revealed conidia ranging from 2 to 3 µm in diameter, with slightly roughened surface ornamentation — features consistent with previous ultrastructural descriptions of this species (Stošić et al., 2020; Sun et al., 2020). Phialide architecture, as observed by SEM, exhibited acerose morphology with biverticillate branching of conidiophores, in full accordance with the taxonomic criteria defined for *Talaromyces* by Samson et al. (2011). The conidia were further characterized as subglobose to ellipsoidal, occasionally sickle-shaped — a feature previously described for members of this section and consistent with the type descriptions (Stošić et al., 2020; Samson et al., 2011). Taken together, the convergence of molecular, phylogenetic, and morphological evidence robustly supports the identification of the isolate as *T. minioluteus*, a species whose lipolytic capabilities are increasingly recognized yet remain underexplored for industrial exploitation.

Future investigations should prioritize the systematic optimization of fermentation parameters — including carbon and nitrogen source composition, temperature, pH, moisture content, and inducer concentration — using statistical experimental designs such as response surface methodology (RSM) or Plackett-Burman design to maximize lipase titers from *T. minioluteus* PCK-01-1124 (Souza et al., 2015; Gupta et al., 2015). Biochemical characterization of the purified enzyme, including determination of optimal temperature and pH, thermal stability profiles, substrate specificity, and kinetic parameters (Km, Vmax), will be essential to evaluate its fitness for targeted industrial applications (Aloulou et al., 2012; Treichel et al., 2010). Beyond production optimization, exploring the performance of this lipase in

specific industrial contexts — such as biodiesel synthesis via transesterification, food flavor modification, pharmaceutical intermediate synthesis, or wastewater treatment — will be critical to establishing its commercial relevance (Jaeger & Reetz, 1998; Mehta et al., 2020).

Conclusions

Palm kernel cake and Crushed date pits, which are rich in oils and lipids and therefore ideal for fungal growth, were explored as a potential source of fungi with lipase-producing capabilities. Seventy-eight fungi were successfully isolated. The PCK-01-1124 isolate showed the highest zone of lipolysis in both phenol red and rhodamine B agar, with a lipase production of 305.87 U/ml, as evaluated by the pNPP assay. The PCK-01-1124 strain was identified as *T. minioluteus*, taxonomically identified, and deposited in GenBank under accession number PV670919.1.

These findings reveal new and interesting insights, proving that locally isolated fungi from PKC can be a valuable source of economical lipase production for biotechnological applications. These findings suggest that fungal populations associated with palm kernel cake possess a greater proportion of lipase-producing strains relative to those from date pits. The PCK-01-1124 propensity for lipase production underscores their suitability for biotechnological exploitation in lipid bioconversion and related applications.

Declarations

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Author Contribution

MEDJEDOUB Sarra: Writing –original draft, methodology, review and editing, data curation, conceptualization, investigation, formal analysis. AIT KAKI - EL HADEF EL OKKI Amel: Writing – original draft preparation.Habiba ZERIZER: Methodology, conceptualization. Faiza BOUGHACHICHE:formal analysis . Safa ELHADJALI: Methodology. All authors reviewed the manuscript.

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Figures

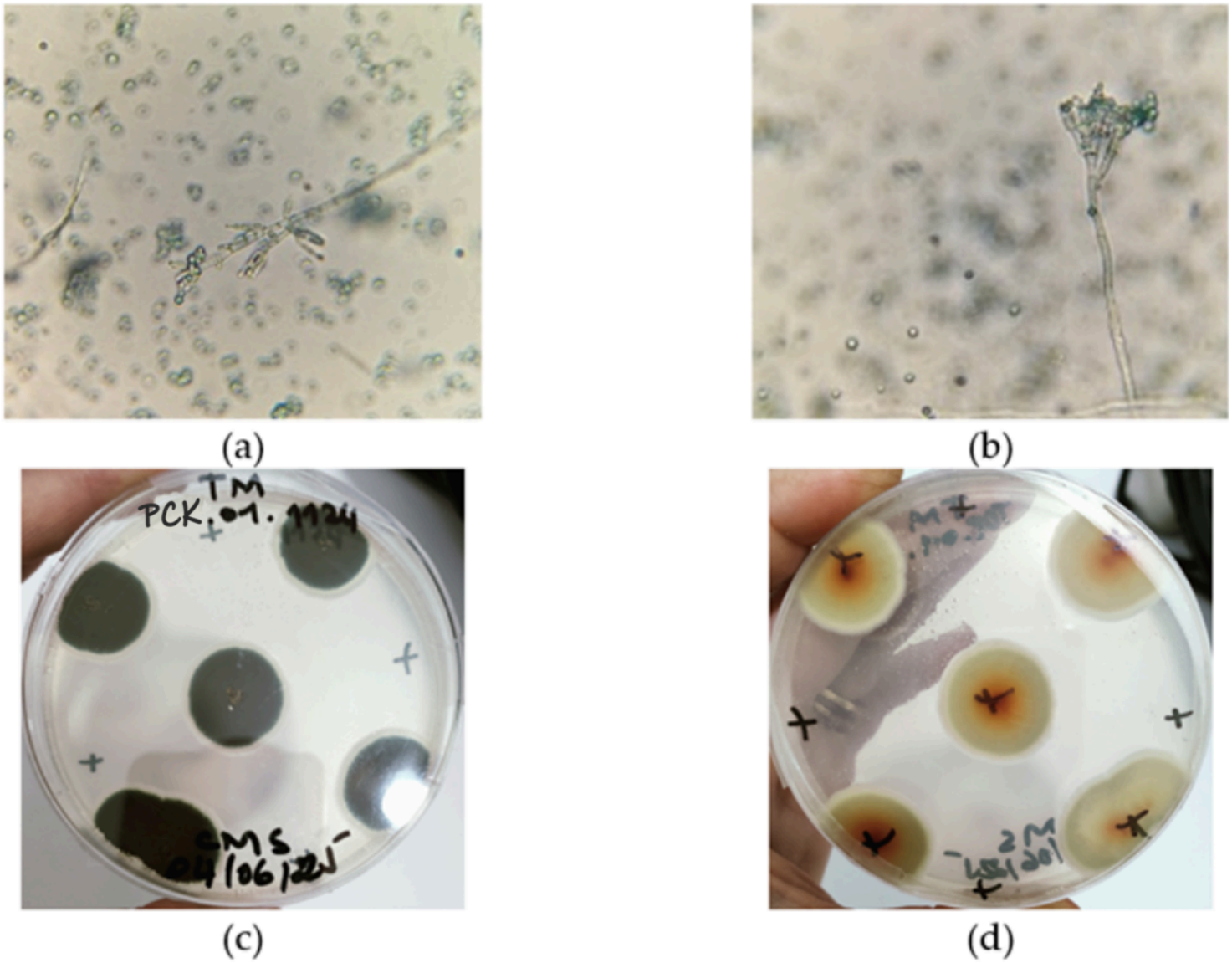


Figure 1

PCK-01-1124 strain characters. (a), (b) microscopic examination with spore X100; (c) obverse; (d) reverse colony on PDA

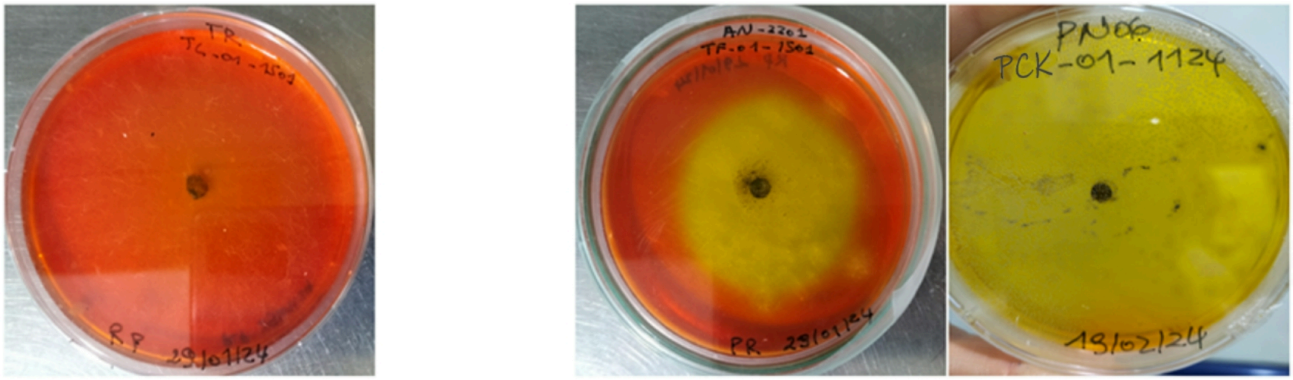


Figure 2

Qualitative analysis of lipase production using agar plate containing olive oil as substrate with Phenol Red as pH indicator: (a) negative strain lipase; (b) left side = positive (+) lipase producer strain, right side = positive (+++) high lipase producer strain.

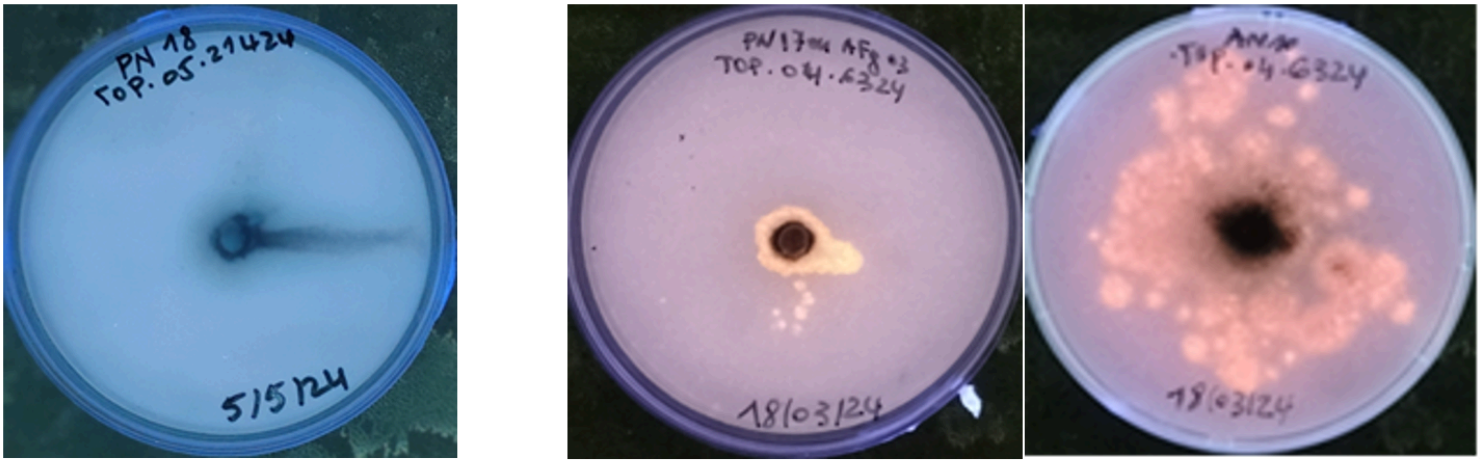


Figure 3

Detection of lipase activity in Rhodamine B plate assays: (a) negative strain lipase; (b) left side = hydrolysis halos of lipase around colonies under UV (+), right side = a highly productive strain of lipase was indicated by the presence of strong lipase activity (+++).

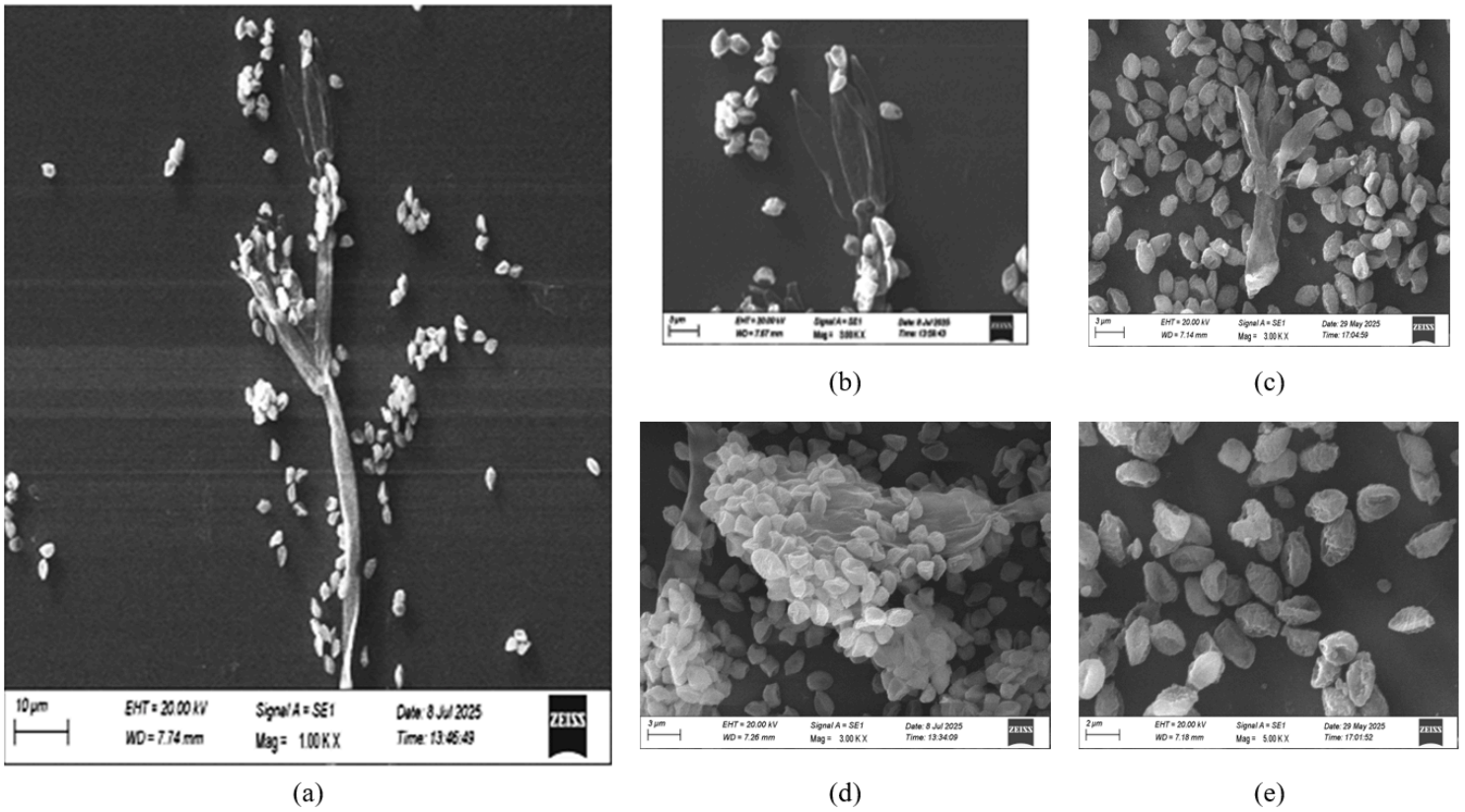


Figure 4

(a) Conidiophores (b, c, d), Phialides and conidia (3000 KX, 3µm), (e) conidia at different magnification (5000 KX, Scale bars applies to a = 3 µm and b = 2 µm)

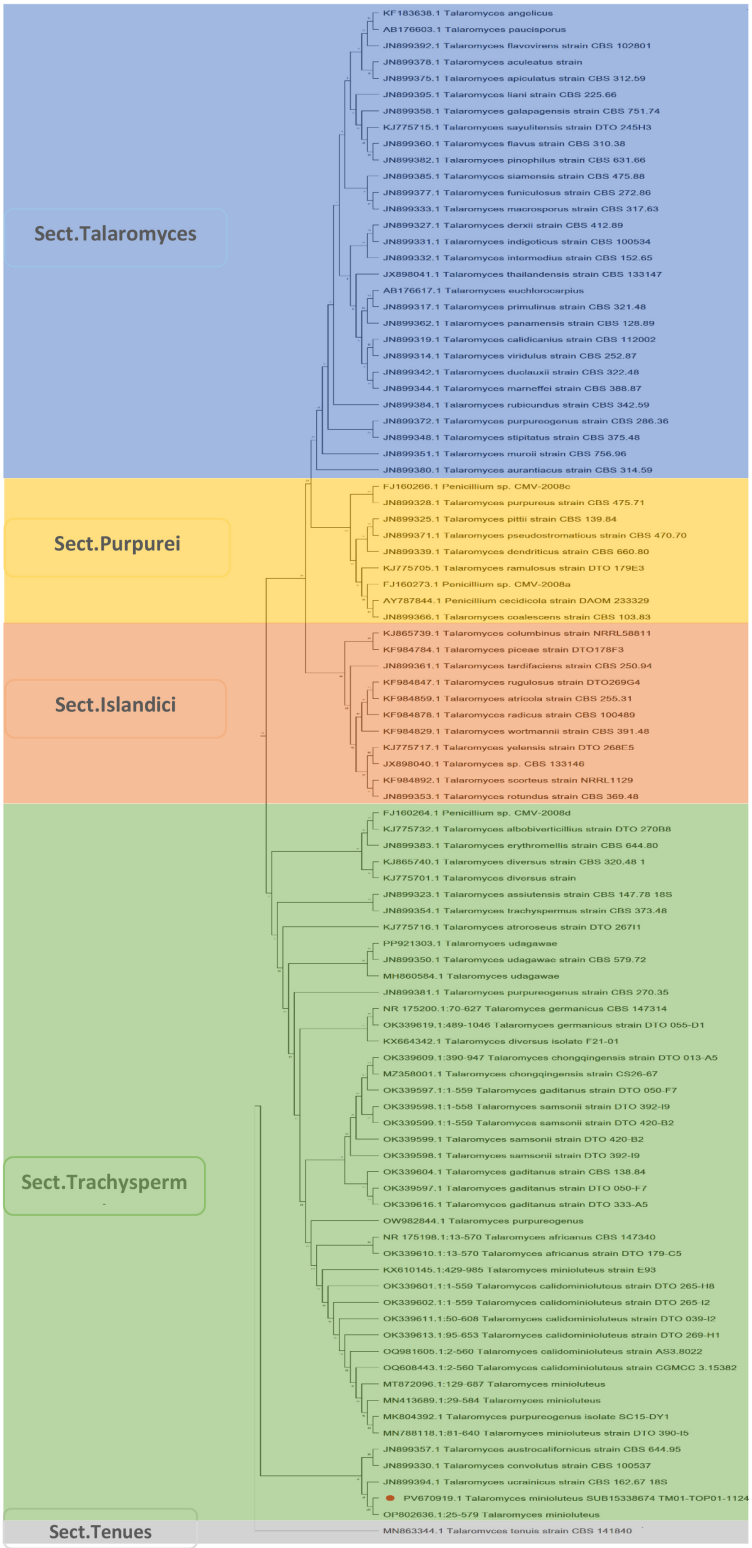


Figure 5

Phylogenetic tree inferred ITS rDNA showing sequence dataset depicting the relationship of the PKC isolate such as *T. minioluteus* and phylogenetically related species.bootsrap replicates 1000

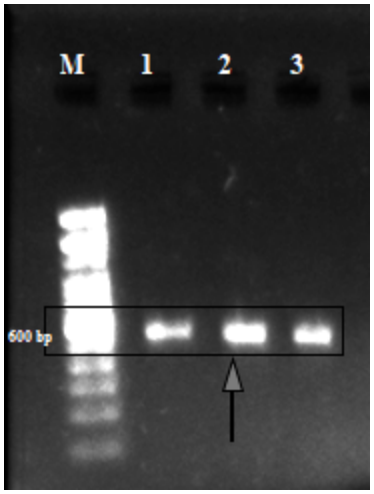


Figure 6

Polymerase chain reaction products obtained from PKC samples following internal transcribed spacer-polymerase chain reaction. Lane 2, PCR product of PCK-01-1124 fungal specie with a specific amplification product of 600 bp;

Supplementary Files

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- PN06Gel.png