Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v47i1.75665

Isolation of fungi potentially producing enzymes of agroindustrial interest: A qualitative analysis of the standardization of the LED light Method

Jean Cristhian da Silva[1*], Gabriel Freitas Borghetti do Carmo[1], Nicole Maria Santos Antunes[2], Caio Roberto Soares Bragança[1] and Vinícius de Abreu D'Ávila[1]

[1]Universidade do Estado de Minas Gerais, Unidade Passos, Avenida Juca Stockler, 1130, 37900-106, Belo Horizonte, Passos, Minas Gerais, Brazil. [2]Faculdade Prominas, Montes Claros, Minas Gerais, Brazil. *Author for correspondence. E-mail: jean.cristhian@uemg.br

ABSTRACT. The biotechnology industry plays a crucial role in the advancement of agronomy, contributing increasingly to each stage of its development. Microbial bioprospecting is a highly diverse field with significant agroindustrial potential, as nature provides a vast range of microorganisms suitable for such applications — including lipase-producing strains. As part of an initiative to establish a fungal collection at the *Universidade do Estado de Minas Gerais* (UEMG) – Passos Unit, 22 fungal isolates were obtained from various natural sources, 17 of which demonstrated lipase production. Fungal isolates with the highest lipolytic activity were derived from wet soil in calf paddocks, oral residues from calves post-nursing, and treated sewage sludge. This study introduces a standardized method using LED light for detecting lipolytic activity — a technique that to the best of our knowledge has not previously been applied to microorganisms. LED light presents a safer alternative to UV light, which can compromise sample integrity and pose health risks to users. This novel approach highlights the diversity of lipase-producing fungi from distinct environmental sources. The study aims to evaluate and qualitatively assess fungal enzymatic activity through this innovative methodology, facilitating the selection of strains with high enzymatic potential for agroindustrial applications.

Keywords: enzymatic activity; bioprospecting; LED light; lipases.

Received on February 11, 2025
Accepted on June 24, 2025

Introduction

Brazilian biodiversity is a valuable resource in the search for new microorganisms that produce enzymes of agroindustrial interest through bioprospecting — a field that aligns productivity with sustainability. Fungi capable of producing lipases have a wide range of applications in industries such as food, leather, cosmetics, detergents, pharmaceuticals, and industrial waste management (Sharma et al., 2001). Among the broad array of enzymes, lipases (triacylglycerol acyl-hydrolases, EC 3.1.1.3) are particularly noteworthy for their ability to catalyze the hydrolysis and synthesis of esters formed from glycerol and long-chain fatty acids. Due to their reaction specificity, stereospecificity, and lower energy requirements compared to conventional methods, microbial lipases have become highly attractive to the biotechnology industry (Verma et al., 2012).

The applicability of these fungi is remarkably diverse. For example, they play a key role in bioremediation efforts (Soares et al., 2011), with increasing use in the decontamination of soils polluted by agroindustrial waste. Lipolytic fungi can also be employed in productive processes such as biodiesel production, using crude enzymatic broth (Batista & Soares, 2023; Bessa et al., 2022). Furthermore, lipase-producing organisms are gaining prominence due to their broad biotechnological potential. Brazil offers significant opportunities for bioprospecting, with a vast reservoir of microbial resources found in both preserved natural environments (Mendonza et al., 2022) and agricultural areas (Fontes et al., 2023).

Brazilian biodiversity offers a solid foundation for identifying novel biocatalysts through traditional microbial selection techniques, which remain central to enzyme discovery across environmental and agroindustrial sectors. Most microbial enzymes are extracellular and are significantly influenced by nutritional and physicochemical factors such as temperature, pH, nitrogen and carbon sources, inorganic salts, agitation, and dissolved oxygen levels (Gupta et al., 2004). Among these, the carbon source is the

primary factor affecting lipase expression, as lipases are inducible enzymes. They are typically produced in the presence of lipids (e.g., oils) or other inducers, including triacylglycerols, fatty acids, hydrolyzable esters, Tweens, bile salts, and glycerol (Sharma et al., 2001). However, nitrogen sources and essential micronutrients must also be carefully optimized to ensure efficient microbial growth and enzyme production (Veerapagu et al., 2013).

Because of the broad applicability of lipid-degrading enzymes — which catalyze a variety of reactions and play a key role in numerous biotechnological processes — research in this field is essential for improving efficiency and identifying promising inoculum sources. Lipolytic activity can vary considerably among microorganisms, highlighting the need to isolate and characterize these organisms while also quantifying their enzymatic activity. Several methods are available for detecting fungal lipolytic activity. One common approach uses the surfactant Tween 80, where the formation of opaque zones around fungal colonies indicates lipase production (Veerapagu et al., 2013).

Alternatively, fluorometric methods can assess specific enzymatic activities. For instance, Rhodamine B forms a complex with fatty acids released during triglyceride hydrolysis by microbial lipases, producing an orange fluorescence under UV light. Initially, samples are exposed to UV light at 365 nm, and enzymatic activity is measured based on the intensity of the orange fluorescence (Lock et al., 2007; Kouker & Jaeger, 1987; Song et al., 2006; Rodrigues, 2016; Zuridah et al., 2011; Winayanuwattikun et al., 2011; Ameri et al., 2015; Oliveira et al., 2014; Boonmahome & Mongkolthanaruk, 2013). However, these methods require caution to ensure operator safety, as UV light is carcinogenic and may induce mutations in microorganisms. Therefore, proper use of personal protective equipment (PPE) is essential (Balogh et al., 2011).

Another promising application of fungi is in Integrated Pest Management through Biological Control. However, as noted by Moras et al. (2016), the effectiveness of an entomopathogenic agent depends on its ability to adhere to and penetrate the insect cuticle, leading to successful host contamination. The production of extracellular enzymes is essential for degrading the insect cuticle, whose outermost layer is wax-based (Gullan & Cranston, 2017).

The greatest expectations for economically and environmentally relevant microorganisms lie in leveraging their biochemical machinery to hydrolyze target substrates (Adrio & Demain, 2014). As a result, the use of microorganisms as 'factories' for biomolecule production has attracted considerable industrial interest, particularly due to their capacity to generate high enzyme yields. Around 75% of industrially utilized enzymes are hydrolases, with lipases (triacylglycerol acyl-hydrolases, EC 3.1.1.3) — members of the serine hydrolase superfamily — standing out as especially important. This class of enzymes holds significant economic value due to its widespread application in various industrial processes (Singh et al., 2017).

The virtually limitless potential of these enzymes justifies the ongoing search for new biomolecules with enhanced properties for industrial applications. Almeida (2020) also discussed the expansion of the agroindustrial sector in relation to 'biofactories' for the production of mycopesticides. The author highlighted the growth of startups in this field, while also pointing out regulatory challenges — particularly those concerning the registration of biological products and compliance with the Biodiversity Law. Strengthening research in this area is essential to overcoming these barriers and fostering sustainable innovation.

Biotechnology is a multidisciplinary field that utilizes organisms, cells, or their components to develop new technologies. Its products are applied across various sectors, including research, agriculture, regenerative medicine, and industry. Modern biotechnology offers innovative tools and solutions to combat diseases, reduce environmental impact, promote the use of cleaner energy as well as develop more efficient, cost-effective agroindustrial processes and products (Melani et al., 2020).

This study aimed to identify environments potentially favorable for the growth of lipase-producing fungi, improve the efficiency of fungal lipolytic detection methods, and quantify enzymatic production — thereby contributing to the bioprospecting of these microorganisms for agroindustrial applications.

Materials and methods

This study was conducted in six sequential stages: (1) selection of lipolytic microorganisms; (2) isolation of the collected microorganisms; (3) detection of lipolytic activity to confirm the fungal strains to be used; (4) standardization of the plate-based method for detecting lipolytic activity; (5) enzymatic extraction; and (6) termination of the hydrolysis reaction, as described by Peil et al. (2016).

Selection of lipolytic fungi

Collection sites were selected based on the types of activities conducted in each area, with a focus on environments with direct or indirect exposure to fatty or oily materials. Samples were transported in Falcon tubes and stored at −70°C. The following sampling sites were included: (1) soil samples: collected from rural areas near the university's academic unit, where intensive mechanized agricultural practices result in petroleum and oil residues, creating a favorable environment for lipid-degrading microorganisms; (2) contact samples from dairy-producing animals: including saliva from buffaloes and calves, as milk is a lipid-rich substance that may promote lipase-producing microbial activity; (3) decanted microbial biomass: obtained from the sewage treatment process through a partnership between the university and the São Paulo State Basic Sanitation Company (SABESP-UEMG); (4) rumen samples: collected from fistulated bovines, whose diverse microbiota is likely to include fungi involved in lipid digestion; (5) insect gut samples: isolated from members of the orders Phasmatodea (herbivores) and Odonata (predators), with the aim of identifying fungi capable of producing lipolytic enzymes.

Isolation of potential lipase-producing fungi

All procedures were carried out under aseptic conditions. To isolate fungi from insect intestines, the digestive tracts were extracted using sterile forceps, disinfected with 70% (v/v) ethanol for one minute, and rinsed with sterile distilled water to remove any ethanol residue. Samples were then homogenized with a sterile glass rod and suspended in 2 mL of saline solution [0.85% (w/v) NaCl] to release adherent microorganisms.

Soil samples were processed by suspending 1 g of soil in 10 mL of sterile saline solution [0.85% (w/v) NaCl]. The same procedure was applied to rumen samples. Contact samples, such as calf saliva or swabs from milk collection buckets, were collected using sterile swabs and transported to the laboratory for processing.

All samples were vortexed for 5 min to break up aggregates and release associated microorganisms. Serial dilutions (10^{-1} to 10^{-6}) were prepared, and 100 µL from each dilution was plated in duplicate on Petri dishes containing appropriate fungal isolation media supplemented with ampicillin.

Growth kinetics and enzymatic activity

To optimize the growth of lipolytic microorganisms, various medium components were tested, including different lipase inducers — Gallo extra virgin olive oil or soybean oil — at concentrations of 2, 4, and 6% (v/v) as well as Triton X-100 and 0.06% (w/v) yeast extract. Samples were subjected to serial dilutions in sterile saline solution [0.85% (w/v) NaCl], and 100 µL aliquots from 10^{-1} to 10^{-3} dilutions were plated on media containing 2% (v/v) Gallo olive oil and 0.1% (w/v) Rhodamine B, supplemented with 0.5 µL of ampicillin to inhibit bacterial growth. Plates were incubated at 25°C for 2 to 10 days. From the collected samples, 22 fungi capable of utilizing olive oil as their sole carbon source were isolated, including 15 filamentous fungi and seven yeasts.

Standardization of plate-based lipolytic activity detection

Lipolytic activity was assessed by observing fluorescent halos around fungal colonies. This study improved the traditional methodology by using a blue LED transilluminator (470 nm wavelength, Model K33-333 – KASVI) as a safer alternative to UV light (365 nm). Fluorescence intensity was used as an indicator of lipolytic activity (Rabbani et al., 2013). A non-lipase-producing strain, Escherichia coli DH5α (Kouker & Jaeger, 1987), was included as a negative control. Fungal strains confirmed to exhibit lipolytic activity were subjected to quantification tests and incorporated into the university's fungal collection, each assigned a unique identification code (Table 1). Samples were stored at −70°C.

Enzyme extraction

Fungal strains that tested positive for lipolytic activity were subjected to enzyme extraction. Potato dextrose agar (PDA) medium was prepared by mixing 5 g of dextrose, 3.7 g of Na_2HPO_4, 2.3 g of citric acid, and distilled water to a final volume of 500 mL. The medium was autoclaved, and 1 mL of ampicillin was added to prevent bacterial contamination. Fungal samples were thawed from −70°C storage, reactivated in 30 mL of PDA medium, and centrifuged at 4°C and 9,000 rpm for 15 minutes. The resulting pellet was resuspended in sterile saline solution (0.85% [w/v] NaCl), and the optical density (OD) was adjusted to 0.15 at 540 nm using a Nanodrop spectrophotometer (Thermo Fisher).

Fungi selected for enzymatic extraction were evaluated for lipolytic activity based on the results of fatty acid titration. The enzymatic activity of each isolate was calculated using the following equation (U mL^{-1}):

Equation:

$$\frac{Va\ -\ Vb\ x\ M\ x\ 1000}{t\ x\ Vc}$$

Where:

- Va: Volume (mL) of NaOH solution used to titrate the sample (specific to each sample)
- Vb: Volume (mL) of NaOH solution used to titrate the blank (6.5 mL)
- Vc: Volume (mL) of the sample used in the reaction (8 mL)
- M: Molar concentration of the NaOH solution (0.05 mol L^{-1})
- t: Reaction time in minutes (30 min)
- 1000: Dilution factor to express the result in U mL^{-1}

Evaluation of lipolytic activity

An extra virgin olive oil emulsion containing 7% Arabic gum (25% olive oil and 75% Arabic gum) and a phosphate buffer solution were prepared and autoclaved to ensure sterility. For each assay, 5 mL of the olive oil emulsion, 2 mL of phosphate buffer (10 mM, pH 7.0), and 1 mL of the fungal enzyme extract were combined. The reaction mixture was incubated at 26°C with agitation at 120 rpm for 30 minutes. The hydrolysis reaction was terminated by adding 10 mL of an acetone–ethanol mixture (1:1, v/v). Fatty acid titration was then performed using 0.05 M NaOH, with phenolphthalein as a pH indicator.

Results and discussion

Among the various sources used for microorganism isolation, this study focused on samples that exhibited growth on media containing extra virgin olive oil, indicating the potential for lipase production by fungi capable of degrading lipids. Of the 22 fungal isolates, 17 displayed orange fluorescence under LED light, confirming lipolytic activity, while five did not produce fluorescent halos. Findings are summarized in Table 1.

Table 1. Origins of the 22 fungal strains from the university fungal collection and their lipolytic activity based on Rhodamine B testing.

Code	Sample Origin	Lipolytic Activity
L 3.1	Buffalo teat residue	+
L 3.1m	Buffalo teat residue	+
L 3.2	Buffalo milk (first draw)	-
L 3.3	Calf feces	-
L 3.4	Buffalo teat residue before milking	-
L 3.5g	Buffalo milk collection bucket residue	+
L 3.6h	Calf mouth residue post-nursing	+
L 3.6l	Calf mouth residue post-nursing	+
L 3.7a	Wet soil from calf paddock	+
L 3.7e	Wet soil from calf paddock	+
L 3.7i	Wet soil from calf paddock	+
L 3.7j	Wet soil from calf paddock	+
L 3.7k	Wet soil from calf paddock	+
L RAb	Tabapuã cattle rumen	+
L RNd	Nelore cattle rumen	+
L 4.1	Odonata intestine	-
L 4.2	Odonata intestine	-
L 4.3a	Phasmatodea intestine	+
L 4.3b	Phasmatodea intestine	+
L 4.3c	Phasmatodea intestine	+
L 4.3d	Phasmatodea intestine	+
L L.1c	Treated sewage sludge	+

Legend: (+) Positive lipolytic activity, (-) Negative lipolytic activity.

Unlike UV light, LED illumination does not damage samples or pose health risks to users, offering improved performance and enhanced safety. This study successfully standardized an LED-based detection method for lipolytic activity, filling a gap in the literature, as no previous reports have applied this approach to microorganisms. In addition to confirming enzymatic activity qualitatively, quantitative assessment is also essential. Peil et al. (2015) evaluated fungal lipolytic activity using Rhodamine B assays (Maciel et al., 2010) and quantified extracellular activity through fatty acid titration.

Although all 22 fungi exhibited growth on lipid-rich media, not all showed positive results in enzymatic activity assays. Plates containing the isolates and Rhodamine B were examined under both LED light and UV light to allow for comparative analysis. Fluorometric methods are widely used for the evaluation and quantification of enzymatic activity. In this study, the blue LED transilluminator (470 nm) effectively excited Rhodamine B, while the UV light used for comparison operated at a wavelength of 365 nm.

Rhodamine B in the medium reacted with fatty acids released during the hydrolysis of triglycerides, a reaction catalyzed by microbial lipases. After 24 hours of incubation, colonies exhibiting lipolytic activity showed orange fluorescence. As noted by Gilham and Lehner (2005), the intensity of fluorescence is directly correlated with the level of lipolytic activity. Figure 1 illustrates this phenomenon, confirming enzymatic activity by the presence of fluorescent orange halos observed under LED illumination.

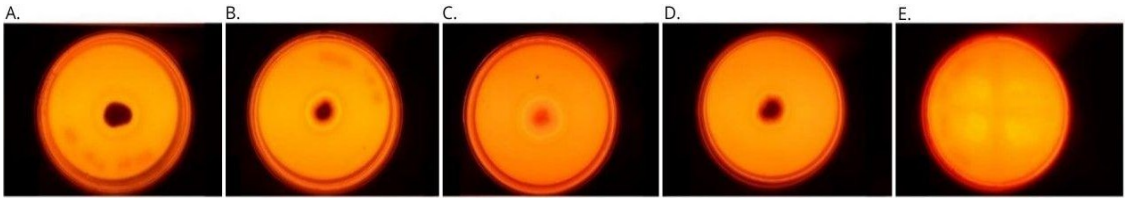

Figure 1. Fungus isolated from the ruminal fluid of Nelore cattle exhibiting lipolytic activity illuminated by LED light (A); fungi isolated from wet soil in calf paddocks showing pronounced enzymatic activity revealed under LED light (B, C); fungus isolated from decanted microbial biomass with visible fluorescence indicative of lipolytic activity exhibited under LED light (D); yeast isolated from wet soil in calf paddocks observed under LED light demonstrating potential for lipase production (E).

Fluorescent halos formed by fungal colonies under LED illumination were used as a qualitative indicator of microbial lipolytic activity and as a criterion for selecting strains with potential for enzyme production. Activity was assessed based on the presence of fluorescent halos surrounding the colony's central growth point. Although this type of qualitative analysis is well documented in the scientific literature, no standardized method using LED light for microbial activity assessment has been reported. Most existing studies rely on UV light for this purpose. As shown in Figure 2, the orange fluorescence observed around fungal colonies results from the hydrolysis of triglycerides and the release of fatty acids, which interact with Rhodamine B in the medium.

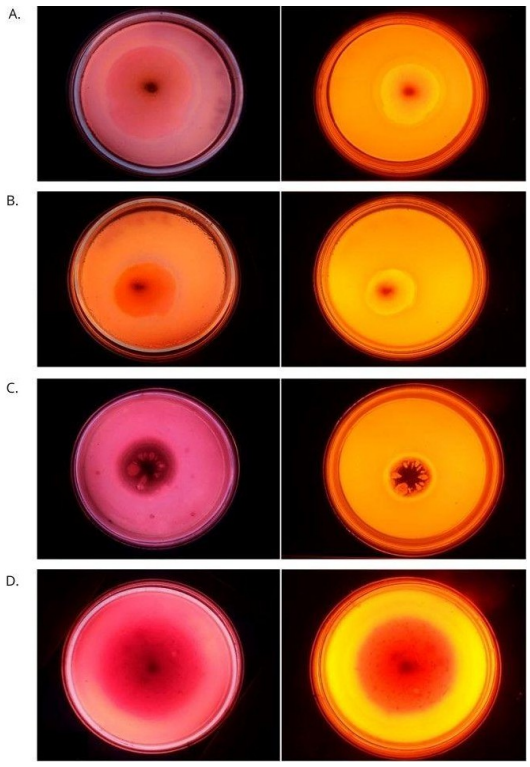

Figure 2. Fungi isolated from wet soil in calf paddocks analyzed under UV transilluminator (left) and LED transilluminator (right), highlighting fluorescence halos from lipolytic activity (A, B); fungus isolated from buffalo milk collection bucket residue under UV (left) and LED (right) transilluminators demonstrating differences in fluorescence detection (C); fungus isolated from Phasmatodea intestine showing lipolytic activity, visualized under UV (left) and LED (right) transilluminators (D).

Using UV irradiation as a reference method, the effectiveness of LED fluorescence for detecting lipolytic activity was evaluated. Isolates exposed to LED light showed positive results indicative of hydrolysis. Following confirmation, 17 fungal isolates demonstrated enzymatic activity (Table 1), showing the ability to degrade olive oil and were subsequently subjected to quantitative analysis. The remaining five isolates likely grew by using alternative nutrients present in the medium, but did not form fluorescent halos, indicating a lack of lipolytic activity. This suggests that these fungi did not produce enzymes capable of degrading the lipid-based carbon source, resulting in no significant hydrolysis or visible indication of enzymatic activity.

The mean values from triplicate enzymatic activity assays were calculated for each sample, providing a reliable measure of the lipolytic activity of the isolated fungi. Table 2 presents the OD readings for each sample, along with the corresponding volume of saline solution used for standardization.

Table 2. Optical density (OD) of each sample and the volume of saline solution required for adjustment.

Sample	Standard OD600	Volume of saline solution required (mL)
L 3.1	3.76	25 ml
L 3.5g	3.66	24.4 ml
L 3.7j	6.32	42.1 ml
L 3.7k	2.28	15.2 ml
L 3.7e	1.21	8 ml
L RNd	1.91	12.7 ml
L 3.7a	2.68	17.8 ml
L 4.3c	1.54	10.2 ml
L 4.3d	1.18	7.8 ml
L 3.6h	1.44	9.6 ml
L 4.3b	1.3	8.6 ml
L 3.7i	1.74	11.6 ml
L 3.6l	3.21	21.4 ml
L 4.3a	1.12	7.4 ml
L RAb	1.3	8.6 ml
L L.1c	1.44	9.6 ml
L 3.1m	4	26.6 ml

Lipolytic activities were quantified using the previously described equation, and the results are presented in Table 3. Higher values correspond to greater enzymatic activity.

Table 3. Titration values and enzymatic activity (U mL^{-1}) results for each sample.

Sample	Titration Values (mL)	Standard Deviation	Result Sample 1 (U mL^{-1})	Result Sample 2 (U mL^{-1})	Result Sample 3 (U mL^{-1})	Average Result (U mL^{-1})
L 3.1	5.8 / 6.1 / 5.8	0.173	-0.15	-0.08	-0.15	-0.13
L 3.5g	4.7 / 5.2 / 5.6	0.450	-0.38	-0.27	-0.19	-0.28
L 3.7j	13.7 / 13.5 / 13.7	0.115	1.5	1.46	1.5	1.49
L 3.7k	8.8 / 8.4 / 9	0.305	0.48	0.4	0.52	0.47
L 3.7e	15.1 / 14.8 / 15.2	0.208	1.79	1.73	1.81	1.78
L RNd	13.6 / 13.1 / 13	0.321	1.48	1.38	1.35	1.4
L 3.7a	11 / 10.5 / 9.3	0.873	0.94	0.83	0.58	0.78
L 4.3c	8.8 / 8.7 / 8.5	0.152	0.48	0.46	0.42	1.36
L 4.3d	8.9 / 7.8 / 9.1	0.7	0.5	0.27	0.54	0.44
L 3.6h	27.7 / 25.8 / 24.6	1.563	4.42	4.02	3.77	4.07
L 4.3b	5.4 / 4.9 / 4.4	0.5	-0.23	-0.33	-0.44	-0.33
L 3.7i	3.8 / 4.5 / 4.7	0.472	-0.56	-0.42	-0.38	-0.45
L 3.6l	9.2 / 8.6 / 8.5	0.378	0.56	0.44	0.42	0.47
L 4.3a	3.5 / 4.3 / 4.4	0.493	-0.63	-0.46	-0.44	-0.51
L RAb	11.8 / 12.2 / 12	0.2	1.1	1.19	1.15	1.15
L L.1c	23.1 / 19.1 / 19.6	2.179	3.46	2.63	2.73	2.94
L 3.1m	11.5 / 10.7 / 10.9	0.416	1.04	0.88	0.92	0.95

The enzymatic activity of each isolate was determined based on individual sample values, and the mean of triplicate measurements was calculated (Table 3). This analysis enabled the selection of fungal strains with the highest enzymatic activity for potential agroindustrial applications. The results showed that fungi isolated from wet soil in calf paddocks (L 3.7e), calf oral residue post-nursing (L 3.6h), and treated sewage

sludge (L L.1c) exhibited the highest enzymatic activity among the 17 lipase-producing isolates. These findings are consistent with those reported by Rodrigues et al. (2016), who successfully isolated lipase-producing fungi from domestic greasy waste and soil exposed to organic residues — environments recognized as favorable for fungal growth and enzymatic expression.

Based on macroscopic and morphological analyses of the isolates, several genera and species were identified (Table 4).

Table 4. Morphological and physiological characteristics of filamentous fungi and yeasts isolated at UEMG.

Isolate	Colony color and texture	Topography	Diffusible pigment	Lipolytic activity	Genus suggestion	Additional observations
L 3.7j	Gray center with white-pinkish halo; velvety	Flat	Not apparent	Moderate to strong	*Penicillium* or *Cladosporium*	Wide and clear halo
L 3.7k	Radially sectorized pigmentation with dark center	Central radial depression	Not evident	Positive	*Aspergillus* or *Talaromyces*	Growth in 'ray' pattern
L 3.7e	Dark green center with white-beige ring; dense and velvety	Raised center	Not detectable	Positive	*A. fumigatus* or dark *Penicillium*	Visible whitish halo
L RNd	Olive to dark green center; velvety texture	Flat with dense center	Not visible	Slight to moderate	*Aspergillus* (section *Nigri*)	Compact colony with well-defined edges
L 3.7a	Olive-brown center with light beige halo; cottony texture	Slightly raised	Absent	Weak to moderate	*Trichoderma* or *Penicillium*	Lighter halo surrounding the colony
L 4.3c	Dark (moss green) center; dense and velvety	Low, uniform	Absent	Moderate	*Aspergillus* sp.	Well-defined clear halo
L 3.6h	Ochre-yellow center with white velvety halo	Raised at the center	Absent	Positive	*Aspergillus flavus* or *Talaromyces*	Characteristic yellow pigmentation
L 3.7i	Gray center with white edge; fine, velvety texture	Flat, diffuse margins	Absent	Slight to moderate	*Cladosporium* or *Fusarium*	Diffuse growth
L 3.6l	Grayish-green center with white edge; cottony texture	Irregular, lobed	Absent	Likely positive	*Penicillium* sp.	Lobed margin and centrifugal growth
L 4.3a	Pale with pinkish tones; dry, rough texture	Irregular with cracks	Orange pigment	Negative or weak	*Fusarium* or *Talaromyces*	Extensive colony, rough surface
L RAb	Black center with olive halo; compact and velvety texture	Flat with radial depression	Absent	Moderate	Suggestive of *Aspergillus niger*	Discreet clear halo around the colony
L L.1c	Green to black center with clear ring; velvety texture	Slightly raised	Not apparent	Moderate to strong	*Aspergillus* sp.	Clear halo surrounding the colony

The sample collection sites showed substantial accumulation of lipid residues. In environments such as calf oral residue post-nursing and wet soil from calf paddocks, the presence of lipids is primarily attributed to continuous exposure to milk. In contrast, treated sewage sludge contains high concentrations of organic waste, serving as a significant lipid source. Over time, these lipid-rich environments have naturally selected for lipase-producing microorganisms. Given their origin, these fungi show promising potential for bioremediation applications aimed at decontaminating soils polluted by agroindustrial waste (Soares et al., 2020). On the other hand, fungi isolated from buffalo teat residue, milk collection bucket residue, and Phasmatodea intestines showed the lowest performance, with negative results indicating an absence of lipolytic enzymatic activity.

Conclusion

Samples L 3.7e and L 3.7j (wet soil from calf paddocks), L 3.6h (calf oral residue post-nursing), and L L.1c (treated sewage sludge) exhibited the highest enzymatic activity, identifying these environments as favorable for the development of lipolytic fungi. Additionally, this study demonstrates that using LED light for the detection of lipolytic activity in microorganisms is a novel and safe approach for the isolation and selection of lipase-producing fungi on solid media. This method ensures greater safety for both researchers and microbial cultures compared to traditional UV-based techniques.

Acknowledgements

The authors would like to thank the *Universidade do Estado de Minas Gerais* (UEMG) for the PQ/UEMG (01/2021) scholarship awarded to Vinícius de Abreu D'Ávila, and the Institutional Research Support Program (PAPq-UEMG) for the scholarships granted to Jean Cristhian da Silva and Gabriel Freitas Borghetti do Carmo.

References

Adrio, J. L., & Demain, A. L. (2014). Microbial enzymes: Tools for biotechnological processes. *Biomolecules, 4*(1), 117-139. https://doi.org/10.3390/biom4010117

Almeida, J. E. M. (2020). Biofábricas para produção de micopesticidas no Brasil: Oportunidades de negócio e inovações. *Brazilian Journal of Animal and Environmental Research, 3*(3), 2444-2557. https://doi.org/10.34188/bjaerv3n3-162

Ameri, A., Shakibaie, M., Amirpour-Rostami, S., Ameri, A., Adeli-Sardou, M., Khazaeli, P., & Forootanfar, H. (2015). Partial purification and characterization of a thermoalkalophilic lipase originated from *Bacillus atrophaeus* FSHM2 and its application for ester synthesis. *Biotechnology, 14*(4), 154-164. https://doi.org/10.3923/biotech.2015.154.164

Balogh, T. S., Velasco, M. V. R., Pedriali, C. A., Kaneko, T. M., & Baby, A. R. (2011). Proteção à radiação ultravioleta: Recursos disponíveis na atualidade em fotoproteção. *Anais Brasileiros de Dermatologia, 86*, 732-742. https://doi.org/10.1590/S0365-05962011000400016

Batista, B. N., & Soares, C. E. V. F. (2023). Microrganismos produtores de lipases para uso na produção de Biodisel. *Revista OWL (OWL Journal) – Revista Interdisciplinar de Ensino e Educação, 1*(2), 413-427. https://doi.org/10.5281/zenodo.8361746

Bessa, D. H. R. F., Flumignan, D. L., Souza, A. O., Gonçalves, M. C. M., & de Souza Castro, C. F. (2022). Obtenção de caldo bruto enzimático a partir de fungos lipolíticos para a produção de ésteres metílicos. *Revista em Agronegócio e Meio Ambiente, 15*(3), 1-13. https://doi.org/10.17765/2176-9168.2022v15n3e9279

Boonmahome, P., & Mongkolthanaruk, W. (2013). Lipase-producing bacterium and its enzyme characterization. *J Life Sci Technol, 1*(4), 196-200. https://doi.org/10.12720/jolst.1.4.196-200

Fontes, A. L., Pimentel, L. L., Soares, A. M. S., do Rosário Domingues, M., Rodríguez-Alcalá, L. M., & Gomes, A. M. (2023). Study of the viability of using lipase-hydrolyzed commercial vegetable oils to produce microbially conjugated linolenic acid-enriched milk. *Food Chemistry, 413*, 135665. https://doi.org/10.1016/j.foodchem.2023.135665

Gilham, D., & Lehner, R. (2005). Techniques to measure lipase and esterase activity in vitro. *Methods, 36*(2), 139-147. https://doi.org/10.1016/j.ymeth.2004.11.003

Gullan, P. J., & Cranston, P. S. (2017). *Insetos: Fundamentos da entomologia* (5a ed.). Editorial ROCA.

Gupta, R., Gupta, N., & Rathi, P. J. A. M. (2004). Bacterial lipases: An overview of production, purification and biochemical properties. *Applied Microbiology and Biotechnology, 64*, 763-781. https://doi.org/10.1007/s00253-004-1568-8

Kouker, G., & Jaeger, K. E. (1987). Specific and sensitive plate assay for bacterial lipases. *Applied and Environmental Microbiology, 53*(1), 211-213. https://doi.org/10.1128/aem.53.1.211-213.1987

Lock, L. L., Corbellini, V. A., & Silva, P. V. D. (2007). Lipases produced by yeasts: Powerful biocatalysts for industrial purposes. *Tecno-lógica, 11*(1-2), 18-25.

Maciel, V. F. A., Pacheco, T. F., & Gonçalves, S. B. (2010). *Padronização do uso de corante rodamina B para avaliação de atividade lipolítica em estirpes fúngicas*.

Melani, N. B., Tambourgi, E. B., & Silveira, E. (2020). Lipases: From production to applications. *Separation & Purification Reviews, 49*(2), 143-158. https://doi.org/10.1080/15422119.2018.1564328

Mora, M. A. E., Castilho, A. M. C., & Fraga, M. E. (2016). Fungos entomopatogênicos: Enzimas, toxinas e fatores que afetam a diversidade. *Revista Brasileira de Produtos Agroindustriais, 18*, 335-349.

Oliveira Carvalho, P., Contesini, F. J., Bizaco, R., & Alves Macedo, G. (2005). Kinetic properties and enantioselectivity of the lipases produced by four *Aspergillus* species. *Food Biotechnology, 19*(3), 183-192. https://doi.org/10.1080/08905430500316342 (Referência não foi citada no texto)

Oliveira, T. F. D., Hidálgo, M. R., & Soares Júnior, M. S. (2014). Production of lipase extrated from aqueous waste: Enzymatic activity kinetics. *Ciência e Agrotecnologia, 38*(6), 562-572. https://doi.org/10.1590/S1413-70542014000600005

Peil, G. H., KuSS, A. V., Rave, A. F., Villarreal, J. P., Hernandes, Y. M., & Nascente, P. S. (2016). Bioprospecting of lipolytic microorganisms obtained from industrial effluents. *Anais da Academia Brasileira de Ciências, 88*, 1769-1779. https://doi.org/10.1590/0001-3765201620150550

Rabbani, M., Bagherinejad, M. R., Sadeghi, H. M., Shariat, Z. S., Etemadifar, Z., Moazen, F., & Zaghian, S. (2013). Isolation and characterization of novel thermophilic lipase-secreting bacteria. *Brazilian Journal of Microbiology, 44*, 1113-1119. https://doi.org/10.1590/S1517-83822013000400013

Rodrigues, C., Cassini, S. T., Antunes, P. W., Keller, R. P., & Gonçalves, R. F. (2016). Isolation and selection of lipase-producing fungi based on lipase activity and hydrolytic potential on soybean oil and grease trap scum. *Engenharia Sanitária e Ambiental, 21*, 507-518. https://doi.org/10.1590/S1413-41522016141401

Sharma, R., Chisti, Y., & Banerjee, U. C. (2001). Production, purification, characterization, and applications of lipases. *Biotechnology Advances, 19*(8), 627-662. https://doi.org/10.1016/S0734-9750(01)00086-6

Singh, M., Chandraveer, T. A., & Tripathi, A. (2017). Isolation and screening of lipase producing microorganisms from natural sources. *Indian Journal of Ecology, 44*(1), 19-23.

Soares, I. A., Flores, A. C., Mendonça, M. M., Barcelos, R. P., & Baroni, S. (2020). Fungos na biorremediação de áreas degradadas. *Arquivos do Instituto Biológico, 78*, 341-350. https://doi.org/10.1590/1808-1657v78p3412011

Song, H. T., Jiang, Z. B., & Ma, L. X. (2006). Expression and purification of two lipases from *Yarrowia lipolytica* AS 2.1216. *Protein Expression and Purification, 47*(2), 393-397. https://doi.org/10.1016/j.pep.2006.02.007

Veerapagu, M., Narayanan, A. S., Ponmurugan, K., & Jeya, K. R. (2013). Screening selection identification production and optimization of bacterial lipase from oil spilled soil. *Asian Journal of Pharmaceutical and Clinical Research, 6*(3), 62-67.

Verma, N., Thakur, S., & Bhatt, A. K. (2012). Microbial lipases: Industrial applications and properties (a review). *International Research Journal of Biological Sciences, 1*(8), 88-92.

Winayanuwattikun, P., Kaewpiboon, C., Piriyakananon, K., Chulalaksananukul, W., Yongvanich, T., & Svasti, J. (2011). Immobilized lipase from potential lipolytic microbes for catalyzing biodiesel production using palm oil as feedstock. *African Journal of Biotechnology, 10*(9), 1666-1673. https://doi.org/10.5897/AJB10.1802

Zuridah, H., Norazwin, N., Siti Aisyah, M., Fakhruzzaman, M. N. A., & Zeenathul, N. A. (2011). Identification of lipase producing thermophilic bacteria from Malaysian hot springs. *African Journal of Microbiology Research, 5*(21), 3569-3573. https://doi.org/10.5897/AJMR11.777