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Lipolytic Potential of an Endophytic Fungus Isolated from the Medicinal Plant *Nyctanthes arbor-tristis*

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Abstract:
Background: *Endophytic fungi are an underexplored source of extracellular enzymes, including lipases, which have wide applications in agriculture, industry, and medicine. This study screened an endophytic fungus from the medicinal plant Nyctanthes arbor-tristis for lipolytic activity.*
Materials and Methods: *The endophytic fungus TPHS1(1) was isolated from the stem of Nyctanthes arbor-tristis collected from Jabalpur, Madhya Pradesh, India, in March 2024. Plant segments were surface sterilized and cultured on potato dextrose agar. Lipolytic activity was screened on Rhodamine B agar and Spirit Blue agar, incubated at 30 °C for 7 days.*
Results: *On Rhodamine B agar, the isolate produced orange fluorescence, which was high on day 3, moderate on day 5, and low on day 7. On Spirit Blue agar, the colony diameter increased from 11 mm to 31 mm and the hydrolysis zone from 14 mm to 40 mm between days 3 and 7, alongside a progressive fading of the blue coloration of the medium.*
Conclusion: *TPHS1(1) showed lipolytic activity on both media, indicating its lipase-producing potential. Quantitative enzyme assays and molecular identification are needed.*
Keywords: Endophytic fungus, Lipase, Rhodamine B Agar, Spirit Blue Agar, Nyctanthes arbor-tristis

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I. Introduction

Plants are a great reservoir of endomycoflora. In 1866, De Bary introduced the concept of endophytes[6]. In ecological literature, the concept of endophytes gained global attention when, in the 1970s, it was discovered that alkaloids synthesized by the fungal endophyte genus *Balansia* (*Ascomycota*) were linked to cases of USA and New Zealand livestock poisoning[4]. Endophytes generally include microbes such as fungi, bacteria, and actinomycetes. These microbes colonise inside the host plant tissues without producing any noticeable harm to the plant, and they live a part of their life span in association with the host plant and stay symptomless. This way, both support each other[13].

Endophytic fungi are a vital and heterogenous group of fungi. These fungi includes ascomycetes and anamorphic fungi[10]. According to fossilised logs, it is suggested that the relationship between plants and endophytes is approximately 400 million years old. Moreover, studies showed that during this period, fungal endophytes have developed various distinct biosynthetic pathways and metabolic activities to produce unique and noble compounds[7]. Numerous investigations into endophytic fungi have revealed their ability to produce extracellular enzymes, including amylase, lipase, cellulase, protease, and laccase, which aid in nutrient enrichment and may enhance plant defense. These hydrolytic enzymes offer a wide range of potential applications across various fields such as agriculture, industry, and medicine. Among these, lipase (EC 3.1.1.3) is a prominent biocatalyst that breaks down triacylglycerols into smaller components[8].

Lipases are vital enzymes that can function with many substances. They are stable and effective in organic solvents. These enzymes are commonly used in producing valuable products like medicines, agricultural chemicals, and natural substances. They can help with different chemical reactions independently. Fungi are often used to produce large amounts of lipases because of their high enzyme yields. However, endophytic fungi remain underexplored as potential lipase sources, even though they are found in many plants [9,2]. Therefore, the present study investigates the lipolytic activity of an identified endophytic fungal isolate, TPHS1(1), recovered from the stem of the therapeutic plant *Nyctanthes arbor-tristis*.

II. Materials and Methods

1. Study Site: The endophytic fungus was isolated from *Nyctanthes arbor-tristis* located at 23.1797° N, 79.9362° E, Jabalpur, Madhya Pradesh, India. The plant sampling was done during the early summer season (March) in the year 2024.

2. Isolation Method: The plant samples were rinsed with running tap water to remove debris and microbes from the surface. Under laminar airflow, the plant samples were cut into small segments (3-4 mm). Surface sterilization was performed by following the steps: placing them in 4% sodium hypochlorite solution (30 s), 70% ethanol (10 s), and finally in autoclaved distilled water. Thereafter, the plant samples were dried on sterilized blotting paper. Then, plant segments were inoculated onto PDA plates and incubated at 26 ± 2 0C for 7 days. The fungal colony that appeared from the trimmed edges was selected and purified on PDA to obtain a pure culture[1].

3. Screening for Lipolytic Enzyme Production: Lipase activity of the endophytic fungal isolate TPHS1(1) was screened on 2 different agar plate media. The first growth medium was Rhodamine B agar containing 4 g yeast extract, 20g Agar, 8g Peptone, 3g NaCl, 0.1g Rhodamine B, 30ml Triton X-100, 1000ml Distilled Water. Lipolytic activity was observed through the presence of orange fluorescence when examined under UV light at 350 nm[3]. The second growth medium was Spirit Blue agar medium containing 32.15 g Spirit Blue agar medium (Himedia), 30 ml tributyrin, and 1000ml Distilled Water. Here, the lipolytic activity was indicated by a halo formation and the discoloration of the medium from blue to transparent around the fungal colony[12]. The inoculated Petri plates were incubated at 30 ^{0}C for 7 days.

III. Results

Certain dyes serve as indicators for lipase activity when added to solid media. In these media, the interaction between lipase and the lipid substrate leads to the breakdown of the substrate, resulting in the release of free fatty acids. Consequently, this release alters the pH of the media, which changes its color and thus signifies the presence of lipase activity[11].

One example of a dye is Rhodamine B. The Rhodamine B agar assay is an effective method for detecting microbial lipase activity. This activity can be observed under UV light at 350 nm, indicated by the orange fluorescence[5]. In this study, the isolated TPHS1(1) demonstrated lipolytic activity on Rhodamine B agar, indicated by the presence of an orange fluorescence (Table 1) when examined under UV light (350 nm). It was observed that on the 3rd day, fluorescence was highest. Then by the 5th day, it decreased to a moderate level and further decreased to a low level by the 7th day (Figure 1). Thus, as the incubation time increased, the fluorescence intensity slowly declined. Similar results were reported by Kumar et al., where 28 fungi were obtained from various natural substrates. Out of these 28, 11 fungi demonstrated lipase activity, which was identified by the presence of orange fluorescence when viewed under UV light[11].

Table 1. Fluorescence intensity of TPHS1(1) on Rhodamine B agar

Incubation (days)	Fluorescence intensity
3	High
5	Moderate
7	Low

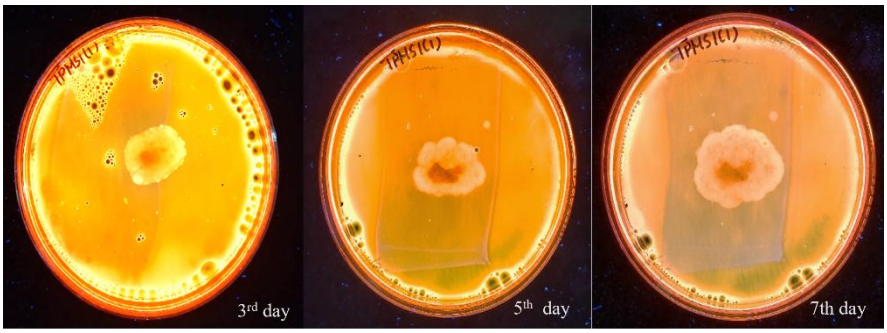


Figure 1. Fluorescence Intensity of TPHS1(1) on Rhodamine B Agar at Different Incubation Periods

Another example of a dye is spirit blue. The Spirit Blue agar medium also effectively detects microbial lipase activity by the concept of colour alternation. In the case of spirit blue agar medium, the fungal isolate TPHS1(1)showed steady growth and formed a clear hydrolysis zone around the colony (Table 2). On the 3rd day, the fungal colony diameter was 11 mm, while the hydrolysis zone was 14 mm. By the 5th day, the colony increased to 19 mm, and the zone to 25 mm. On the 7th day, the colony reached a diameter of 31 mm, with a zone of 40 mm. The medium also changed its color with the incubation time. On the 3rd day, the medium color was blue, but

with a clear zone around the colony. By day 5th the blue started to turn transparent, and by day 7th almost no blue colour remained, with a distinct clear zone (Figure 2). This gradual change in colour indicates the hydrolysis of tributyrin and the liberation of fatty acids. Similarly, Simonovicova et al. studied lipase activity of fungi on Spirit Blue Agar medium. All fungi showed positive results, and most of the isolated fungi were *Aspergillus* species, followed by *Penicillium* and *Rhizopus*[12].

Table 2. Lipase activity of TPHS1(1) on Spirit Blue agar

Incubation (days)	Colony diameter (mm)	Hydrolysis zone (mm)	Zone/colony ratio	Medium appearance
3	11	14	1.27	Blue with clear zone
5	19	25	1.32	Blue turning white, clear zone
7	31	40	1.29	Negligible blue, clear zone

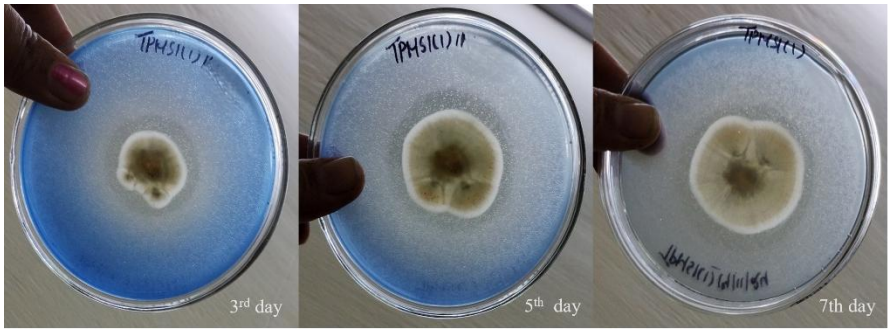


Figure 2. Lipase Activity of TPHS1(1) on Spirit Blue Agar at Different Incubation Periods

The two media report different aspects of lipase activity and provide supporting information. On Rhodamine B agar medium, strong fluorescence was observed on the 3rd day of incubation, indicating that this medium can be useful for rapid qualitative screening of lipase production by microbes. On Spirit Blue agar medium, the hydrolysis zone and fungal colony continued to increase with incubation time, and the blue color of the medium gradually faded. Thus, the enzymatic activity can be observed until the 7th day. As the two media include different lipid substrates and depend on different detection methods, positive results on both media provide stronger evidence of lipolytic activity than either result alone. However, these agar plate assays are primary screening methods, and further measurement of enzyme activity in the culture filtrate by titrimetric or spectrophotometric assays is required. Moreover, molecular identification of the isolate is essential.

IV. Conclusion

This study examined the endophytic fungal isolate TPHS1(1), recovered from the stem of *Nyctanthes arbor-tristis*, for lipolytic activity on two agar media. The isolate displayed orange fluorescence on Rhodamine B agar and a clear hydrolysis zone with a declining blue color on Spirit Blue agar, demonstrating extracellular lipase production. In Rhodamine B agar medium, the fluorescence was highest on the 3rd day of incubation and decreased by the 7th day of incubation, while in Spirit Blue agar medium, the hydrolysis zone increased from 14 mm to 40 mm as the colony grew. Rhodamine B agar and Spirit Blue agar both contain different lipid substrates and depend on different detection methods, thus providing a broader understanding of lipase production. Rhodamine B agar is appropriate for fast qualitative screening, whereas Spirit Blue agar allows the observation of enzyme activity for a longer time. A positive outcome on both media provides effective proof of lipolytic activity compared to either medium used individually. However, it is important to note that plate assays serve only as preliminary investigations. For a broader understanding, quantitative measurement of lipase activity in the culture filtrate, along with molecular identification of the isolate, is necessary.

References

[1]. Aharwal RP, Kumar S, Thakur Y, Deshmukh L, Singh S. Evaluation of antibacterial activity of endophytic fungi *Aspergillus japonicus* isolated from *Tridax procumbens* L. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(9):211-216.
[2]. Costa-Silva TA, Souza CR, Oliveira WP, Said S. Characterization and spray drying of lipase produced by the endophytic fungus *Cercospora kikuchii*. Brazilian Journal of Chemical Engineering. 2014;31(4):849-58.
[3]. Elhussiny NI, Khattab AE, El-Refai HA, Moham ored SS, Shetaia YM, Amin HA. Assessment of waste frying oil transesterification capacities of local isolated *Aspergillus* species and mutants. Mycoscience. 2020;61(3):136-44.
[4]. Firaková S, Šturdíková M, Múčková M. Bioactive secondary metabolites produced by microorganisms associated with plants. Biologia. 2007;62(3):251-7.
[5]. Geoffry K, Achur RN. Screening and production of lipase from fungal organisms. Biocatalysis and agricultural biotechnology. 2018;14:241-53.
[6]. Khare E, Mishra J, Arora NK. Multifaceted interactions between endophytes and plant: developments and prospects. Frontiers in microbiology. 2018;9:1-12.

[7]. Li SJ, Zhang X, Wang XH, Zhao CQ. Novel natural compounds from endophytic fungi with anticancer activity. European Journal of Medicinal Chemistry. 2018 ;156:316-43.
[8]. Peepre T, Sandhu SS. Isolation and Screening for Lipase Activity in an Endophytic Fungus Associated with *Nyctanthes arbor-tristis*. IJSREM. 2026;10(10):1-4.
[9]. Rocha KS, Queiroz MS, Gomes BS, Dallago R, de Souza RO, Guimaraes DO, Itabaiana Jr I, Leal IC. Lipases of Endophytic Fungi Stemphylium lycopersici and Sordaria sp.: Application in the synthesis of solketal derived Monoacylglycerols. Enzyme and microbial technology. 2020;142:1-33.
[10]. Selim KA, El-Beih AA, abdel-Rahman T, El-Diwany AI. Biology of endophytic fungi. Current Research in Environmental & Applied Mycology. 2012;2(1):31-82.
[11]. Sharma AK, Sharma V, Saxena J. A review on different screening methods to identify and isolate potent lipase producers.2016;5(12):269-278.
[12]. Simonovicova A, Kraková L, Pangallo D, Majorošová M, Piecková E, Bodoriková S, Dörnhoferová M. Fungi on mummified human remains and in the indoor air in the Kuffner family crypt in Sládkovičovo (Slovakia). International Biodeterioration & Biodegradation. 2015;99:157-64.
[13]. Tripathi A, Pandey P, Tripathi SN, Kalra A. Perspectives and potential applications of endophytic microorganisms in cultivation of medicinal and aromatic plants. Frontiers in plant science. 2022;13:1-21.