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Isolation and Screening for Lipase Activity in an Endophytic Fungus Associated with Nyctanthes arbor-tristis

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Abstract

Endophytic fungi are important sources of extracellular enzymes with potential applications in various industrial processes. The present study aimed to screen the lipase activity of an endophytic fungal isolate, TPS1, obtained from the stem of Nyctanthes arbor-tristis. The fungal isolate was recovered using standard surface sterilization and culturing techniques and purified on potato dextrose agar (PDA). Morphological identification was verified by macroscopic colony features and by microscopic examination using lactophenol cotton blue staining. Lipase activity was evaluated using Spirit Blue agar medium supplemented with olive oil as the substrate. The inoculated plates were incubated at 30°C for seven days and observed for fungal growth and changes in medium colour. No growth or colour change was observed on the first day. The colony diameter reached 11 mm on day three and 22 mm on day five, when the medium began changing colour. By day seven, the colony diameter reached 24 mm, and the medium became transparent. These observations suggest potential lipase activity in isolate TPS1, warranting further quantitative enzyme analysis.

Keywords: Endophytic fungi, Lipase activity, Spirit blue, Extracellular enzymes, Plants

1. Introduction

Plant-associated fungi that colonize the internal plant tissues asymptomatically are known as endophytic fungi. The presence of these microorganisms is documented in various plant species, indicating their reciprocal relationship with the host plant that safeguards the host from various biotic and abiotic stresses and promotes plant growth. Further, these fungi are highly diverse and serve as a rich reservoir of bioactive compounds [9]. Endophytic fungi often produce compounds similar to those produced by their host plant [4].

Various studies on endophytic fungi have shown their potential for extracellular enzyme production, including amylase, lipase, cellulase, protease, and laccase, which help in nutrient addition and may contribute to plant protection [2]. These hydrolytic enzymes represent a broad range of applications in different areas such as agriculture, industry, and medicine. Among them, lipase (EC 3.1.1.3) is a well-known biocatalyst that hydrolyzes triacylglycerols into small units. Fungal endophytes are an important source of lipase enzymes that have various industrial roles, including biodiesel production.

Biodiesel is a renewable fuel which can replace diesel fuel obtained from fossil fuels. However, its production faces several challenges such as high raw material costs and various environmental, technical and economic issues. Enzymatic transesterification is an expensive process but its advantages have motivated the search for new sources of lipase [5]. Apart from this, fungal endophytes are also recognized to have a possible role in bioremediation since their enzymes have the ability to break down environmental pollutants [2]. Thus, this study aimed to examine the lipase activity of unidentified endophytic fungal isolate TPS1 obtained from Nyctanthes arbor-tristis on spirit blue agar medium with olive oil.

2. Methods and Material

2.1 Isolation

In this study, fungal isolate TPS1 was obtained from the stem of Nyctanthes arbor-tristis. The isolation of fungi was done as described by [4]. The plant sample was washed under running tap water to eliminate microbial load and debris present on the plant surface. Afterward, under aseptic conditions, the sample was trimmed into small segments (3- 4 mm). Surface sterilization of segments was performed by placing them into sodium hypochlorite (3 %) for 1 min, followed by ethanol (70 %) for 1 min, and then rinsed with sterilized distilled water. All the segments were dried on sterilized filter paper. After that, stem segments were placed on potato dextrose agar (PDA) plates and incubated at 26±2 ^{0}C for 7 days. The emerged fungal colony was purified by transferring it to PDA to obtain a pure culture.

2.2 Morphological Identification

The identification of fungal isolates was carried out by examining the colony morphology and microscopic features of the fungal isolates. The macroscopic identification of fungal isolates was done by observing the colony shape, colour, texture, margin, front and reserve colour of colony. These features were studied on PDA medium after incubation. Microscopic examination of the fungal isolates was performed using lactophenol cotton blue staining. The slide was prepared with the help of lactophenol-cotton blue stain where a small drop of stain was poured on a small portion of the fungal culture on the slide, then gently covering it with a cover slip and observing under the microscope, where microscopic features were studied [7,3].

2.3 Screening for Lipase Activity

The lipase activity of endophytic fungal isolate TPS1 was screened on Spirit Blue agar medium (32.15 g Spirit Blue agar medium (Himedia) in 1000 ml distilled water), using olive oil (30 ml) as the substrate. The spirit blue agar medium was autoclaved at 121 ^{0}C for 15 min. After autoclaving, the antibiotic was added to the medium, the mixture was well mixed by stirring, allowed to cool, and then about 15-20 ml was poured into sterilized Petri plates. Once the medium was solidified, a small portion of the fungal culture was placed on the spirit blue agar plates. The inoculated petri plates were incubated at 30 ^{0}C for up to 7 days. The inoculated petri plates were observed for any change in the colour of the medium [8].

3. Result and Discussion

3.1 Morphological Identification

3.1.1 Macroscopic Features

The fungal isolate TPS1 showed rapid growth on PDA. The fungal colony was found to be circular with an irregular, diffuse margin. Its texture was cottony, with aerial mycelial growth. The colour of the front surface was creamy white, and greyish-brown pigmentation was present in the centre. The reverse surface was yellowish cream in colour with brown pigmentation in the centre.

3.1.2 Microscopic Features

The microscopic examination showed long, branched, septate hyphae. Small, oval to ellipsoidal-shaped conidia-like structures were observed.

3.2 Lipase Activity

Lipase activity of microorganisms in a solid agar assay can also be observed using Spirit Blue dye. The Spirit Blue Agar medium is based on the concept of colour alternation. This occurs when the pH of the medium decreases due to the liberation of fatty acids [1]. In this study, the lipase activity of endophytic fungal isolate TPS1 was examined on Spirit Blue agar medium augmented with olive oil as the substrate. On the 1st incubation day, neither fungal growth nor change in medium colour was observed. Fungal growth was observed on the 3rd day of incubation, with a 11 mm colony

diameter, while the medium remained blue. On the 5th day, fungal growth increased to 22 mm and showed lipase activity as the medium started changing its colour. By day 7, the fungal colony diameter reached 24 mm, and the medium became completely transparent, indicating hydrolysis of olive oil by extracellular lipase enzymes produced by the fungal isolate (Fig. 1). Similarly, an investigation examined the lipase activity of 16 strains of fungus named Aspergillus niger with the help of Spirit Blue Agar medium. It was documented that each strain displayed lipase activity, as confirmed by colour alternation of the medium around the colony. Although, strain An–G showed maximum activity, while strains, An–Pop4, An–P, An–L18, An–ZK, and An–SL, showed minimum lipase activity [6].

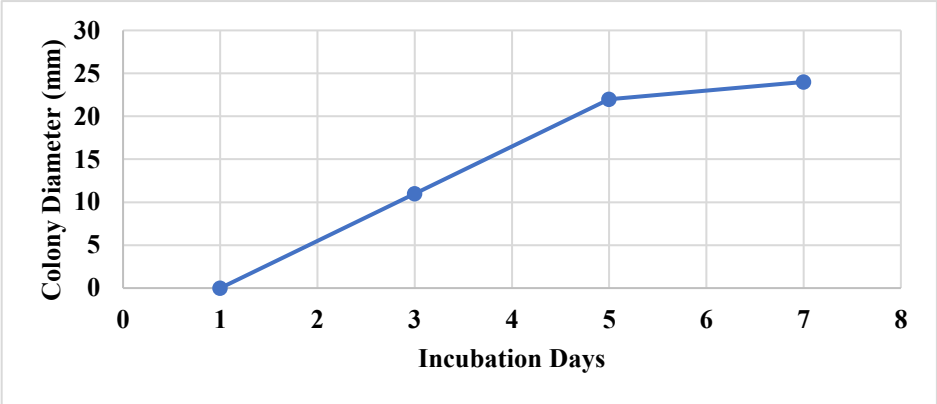


Figure 1. Day-wise growth of Fungal isolate TPS1

4. Conclusion

The current investigation highlights the lipase activity of endophytic fungal isolate TPS1 recovered from the stem of Nyctanthes arbor-tristis. The isolate was fast growing on PDA, and macroscopic and microscopic traits were studied. The isolate was screened for lipase activity using Spirit Blue agar supplemented with olive oil, and changes in the medium were observed during incubation. The colony diameter increased from 11 mm on day three to 24 mm on day seven, while the medium became completely transparent on the 7th day. These findings suggest that isolate TPS1 produces extracellular lipase capable of hydrolyzing olive oil. These results indicate that Nyctanthes arbor-tristis may serve as a possible source of lipase-producing fungal endophytes that may be useful for biodiesel production and bioremediation.However, this research is a qualitative assessment based on a single medium, and the isolate remains unidentified. To confirm its activity and evaluate its biotechnological potential, molecular identification, testing on other lipase-detection media, and quantitative enzyme assays are necessary.

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