



Isolation, Identification, and Characterization of Soil Fungi for Extracellular Enzyme Production

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ABSTRACT

Soil fungi represent a diverse group of microorganisms with significant potential for industrial enzyme production. The present study focuses on the identification, isolation, and characterization of soil fungi capable of producing extracellular enzymes. Soil samples were collected from diverse ecological niches and subjected to serial dilution and spread plate techniques for fungal isolation using selective media such as Potato Dextrose Agar (PDA). Distinct fungal colonies were purified and identified based on morphological, microscopic, and molecular characteristics. Preliminary screening for enzyme production was conducted using plate assay methods to detect amylase, cellulase, protease, and lipase activities. Positive isolates were further subjected to quantitative enzyme assays under optimized growth conditions. The study successfully identified several potent fungal isolates demonstrating significant enzymatic activity, indicating their potential for industrial-scale enzyme production. These findings underscore the ecological and biotechnological significance of soil fungi as sustainable bio resources for applications in food processing, textile, detergent, pharmaceutical, and biofuel industries.

Keywords: Soil-fungi, Enzymes, Bio-resources

INTRODUCTION

Fungal extracellular enzymes are proteins secreted by fungi beyond their cell walls into the surrounding environment, where they catalyze the hydrolysis of complex organic substrates such as polysaccharides, proteins, and lipids into simpler, absorbable monomers. This extracellular secretion allows fungi to access nutrients from insoluble materials that cannot penetrate cell membranes, distinguishing them from intracellular enzymes confined within the hyphae. These enzymes play a pivotal role in fungal physiology and evolution, enabling fungi to thrive as primary decomposers in nutrient-scarce terrestrial ecosystems by breaking down recalcitrant biopolymers like lignocellulose. Their activity underpins global biogeochemical cycles, particularly the carbon and nitrogen cycles, by facilitating the mineralization of organic matter and preventing nutrient lockup in undecomposed biomass. For instance, fungi and bacteria together decompose over 90% of terrestrial plant

litter that escapes herbivory.

Evolutionarily, this saprotrophic strategy has allowed fungi to colonize diverse niches since their emergence in aquatic environments around 1 billion years ago and colonization of terrestrial habitats over 400 million years ago, contributing to soil fertility and ecosystem resilience.

The large majority of the 80,000+ fungal species described are likely to occur in soil environments at some stage in their life cycle (Bridge and Spooner, 2001). Soil fungi are vital for ecosystem functioning by facilitating degradation of organic matter, nutrient release, and soil carbon sequestration, especially in boreal forests (Clemmensen et al., 2013). Fungal activity is most prominent during early stages of decomposition of leaves and wood and declines in later stages when bacterial dominance increases.

Fungi utilize both simple and complex substrates through secretion of extracellular enzymes (EEAs), including proteases, cellulases, β -glucosidases, and chitinases (Redlak et al., 2001; Cullings et al., 2008; Zifcakoya et al., 2011). These enzymes degrade plant proteins, cellulose, hemicellulose, starch, and animal-derived compounds such as chitin. In soils, phosphatases secreted by plants and microorganisms play a critical role in the phosphorus cycle by converting organic phosphorus into inorganic forms available for uptake (Marcote et al., 2001; Caldwell, 2005; Bolan, 1991; Cosgrove, 1977).

Ligninolytic enzymes such as laccases and polyphenol oxidases are involved in the degradation of lignin and various xenobiotic aromatic compounds (Pointing, 2001; Jarosz-Wilkolazka et al., 2002; Burke RM, Cairney, 2002). Extracellular enzyme activities are also important indicators of soil biological equilibrium, fertility, and quality, as they participate in nutrient cycling, energy metabolism, and degradation of diverse compounds (Bandick, 1999; Ekenler and Tabatabai, 2003; Rousk et al., 2010).

Advancements in fungal diversity studies have been achieved through next-generation sequencing techniques and traditional observation of fruiting bodies across diverse environments (DeNovio et al., 2004). Soil colloids, typically ranging from 10 nm to 10 μ m in diameter, represent the most reactive fraction of soil and significantly influence its physical and chemical properties. These colloids consist of both organic and inorganic components with varying reactivity (Oyeleke et al., 2010).

MATERIAL AND METHODS

Collection of soil samples:

Soil samples were collected randomly from Chh. Sambhajinagar (Aurangabad MH) district. The soil samples were collected from a depth of about 15 cm with the help of sterilized spatula in sterile polyethnec bags and well labeled with site of collection. (Oyelekee et al., 2010)

Isolation and purification of fungi:

Fungi from the collected soil samples were isolated using the serial dilution agar plate method as described by Kader et al., (1999). 1 gram of each soil samples were mixed with 9ml of sterile water and serial dilutions method were performed 10¹ to 10⁶ in a sterile laminar air flow chamber. A volume of 0.1 ml from each dilution tube was spread on to potato dextrose agar media (PDA) plates with the help of sterile glass rod (Choudhary and Jain, 2012; Sethi and Gupta, 2015). The inoculated Potato Dextrose Agar (PDA) plates were incubated at 28 °C for a duration of 5 days period. Fungal colonies developed on the Potato Dextrose Agar plates and slants were prepared from these isolated fungal sp. Pure culture done using test tube containing fresh agar slants of PDA media for further studies at very lower temperature.

Identification of fungi

The fungal isolates were made by both the observation of colony features and cellular characteristics using lactophenol cotton blue stain used as the mounting

fluid. The slide observed under the light microscope at 10x and 40x microscopic magnification. Identification of hyphae, spores, sporangiospores of fungi was done by employing the method given by Barnett and Hunter (1972).

Assessment of the Enzymatic Activity of the Isolated Fungi

Different growth media were used for testing the fungal enzymatic activity; PDA medium was used for the activation of all fungal isolates for one week prior to enzyme assessment. Disk was cut from the edge of each fungal isolate using 5 mm cork borer and placed in the middle of the media to check for enzyme activity.

Amylase Enzyme

The capacity to break down starch serves as a criterion for assessing the ability to produce amylase enzymes. The medium contained malt extract and 0.2% soluble starch with a pH of 9. After 3–5 days of incubation, the plates were soaked with an iodine solution, and a yellow region surrounding a colony in a blue medium indicated amylase activity (Ileri et al., 2015).

Cellulase Enzyme

Estimation of cellulase enzyme was made using carboxymethyl cellulose medium (CMC). The composition of this medium per liter were: KCl 0.5 g, K_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $NaNO_3$ 2 g, carboxymethyl cellulose 2g and agar 20 g and distilled water 1 L. The plates were flooded with a 0.2% aqueous solution of Congo red after an incubation period of 7 days at 25 °C, afterward decolorized with

1M NaCl for 15 min. Positive activity was indicated by the presence of yellow zones around the fungal colony; equally negative activity signifies by the red medium (Zifcakova et al., 2011).

Lipase Enzyme

The capability of the purified fungi to secrete lipase enzyme was made on peptone agar medium. The medium contained the following: NaCl 5g, peptone 10g, $CaCl_2 \cdot 2H_2O$ 0.1g, agar 20g, and distilled water 1 L, with a pH of 6.0, and included 1% Tween 20, which was sterilized separately using a 0.45 µm Millipore filter paper before being added to the medium. After incubation period of seven-day at 25 °C, it was prominent that certain fungi showed noticeable precipitation surrounding the colony, attributable to the production of calcium salts from lauric acid released by the enzyme, demonstrating confident lipase activity. Similarly, others did not produce any precipitation, indicating negative activity (Zifcakova et al., 2011).

RESULT AND DISCUSSION

Enzymatic Activity

The enzymatic activity of 21 fungal species was evaluated to determine their ability to produce cellulase, amylase, lipase, and ligninase enzymes. Distinctive variance was noted among the different fungal species concerning their enzymatic abilities. Nearly all fungi showed diverse enzymatic abilities, but at variable amounts, with differing quantities of enzymes produced by each species.

Table 1: Fungal enzymatic activity for cellulase enzyme.

Sr. No.	Fungal species	Cellulase enzyme activity
1	<i>Alternaria chlamydospora</i>	-
2	<i>Aspergillus caespitosus</i>	+++
3	<i>Aspergillus flavus</i>	-
4	<i>Aspergillus fumigatus</i>	-
5	<i>Aspergillus niger</i>	-
6	<i>Aspergillus ochraceopetaliformis</i>	+
7	<i>Aspergillus Pulvericola</i>	+++
8	<i>Aspergillus terreus</i>	-
9	<i>Aspergillus versicolor</i>	-
10	<i>Aspergillus wentii</i>	-
11	<i>Cladosporium herbarum</i>	-
12	<i>Fusarium solani</i>	-
13	<i>Mucor hiemalis</i>	-
14	<i>Penicillium oxalicum</i>	+++

15	<i>Penicillium rubefaciens</i>	+++
16	<i>Rhizopus stolonifer</i>	-
17	<i>Talaromyces atrovirens</i>	+++
18	<i>Trichoderma harzianum</i>	-
19	<i>Talaromyces minioluteus</i>	+++
20	<i>Talaromyces purpureogenus</i>	+++
21	<i>Talaromyces verruculosus</i>	-

- No secretion change; + weak secretion (3-5) mm; ++ medium secretion (5-8) mm; +++ strong secretion (8-11) mm.

Table 2: Fungal enzymatic activity for amylase enzyme.

Sr. No.	Fungal species	Amylase enzyme activity
1	<i>Alternaria chlamydospora</i>	-
2	<i>Aspergillus caespitosus</i>	+++
3	<i>Aspergillus flavus</i>	-
4	<i>Aspergillus fumigatus</i>	-
5	<i>Aspergillus niger</i>	-
6	<i>Aspergillus ochraceopetaliformis</i>	+
7	<i>Aspergillus Pulvericola</i>	+++
8	<i>Aspergillus terreus</i>	+++
9	<i>Aspergillus versicolor</i>	-
10	<i>Aspergillus wentii</i>	-
11	<i>Cladosporium herbarum</i>	-
12	<i>Fusarium solani</i>	-
13	<i>Mucor hiemalis</i>	-
14	<i>Penicillium oxalicum</i>	-
15	<i>Penicillium rubefaciens</i>	-
16	<i>Rhizopus stolonifer</i>	-
17	<i>Talaromyces atrovirens</i>	+++
18	<i>Trichoderma harzianum</i>	-
19	<i>Talaromyces minioluteus</i>	-
20	<i>Talaromyces purpureogenus</i>	+++
21	<i>Talaromyces verruculosus</i>	+++

- No secretion change; + weak secretion (3-5) mm; ++ medium secretion (5-8) mm; +++ strong secretion (8-11) mm.

Table 3: Fungal enzymatic activity for lipase enzyme.

Sr. No.	Fungal species	Lipase enzyme activity
1	<i>Alternaria chlamydospora</i>	-
2	<i>Aspergillus caespitosus</i>	-
3	<i>Aspergillus flavus</i>	-
4	<i>Aspergillus fumigatus</i>	-
5	<i>Aspergillus niger</i>	-
6	<i>Aspergillus ochraceopetaliformis</i>	-
7	<i>Aspergillus Pulvericola</i>	-
8	<i>Aspergillus terreus</i>	-
9	<i>Aspergillus versicolor</i>	-
10	<i>Aspergillus wentii</i>	-
11	<i>Cladosporium herbarum</i>	-
12	<i>Fusarium solani</i>	-

13	<i>Mucor hiemalis</i>	-
14	<i>Penicillium oxalicum</i>	-
15	<i>Penicillium rubefaciens</i>	-
16	<i>Rhizopus stolonifer</i>	-
17	<i>Talaromyces atrovirens</i>	+++
18	<i>Trichoderma harzianum</i>	-
19	<i>Talaromyces minioluteus</i>	+++
20	<i>Talaromyces purpureogenus</i>	+++
21	<i>Talaromyces verruculosus</i>	-

- No secretion change; + weak secretion (3-5) mm; ++ medium secretion (5-8) mm; +++ strong secretion (8-11) mm

This phenomenon can be attributed to the intrinsic enzymatic activity inherent to each fungus, as it is commonly documented that each microorganism shows a distinct enzymatic ability, influenced by its environmental adaptation and secretory capabilities (Al-Harathi, 2025; Zifcakova et al., 2011; Patil et al., 2015).

Cellulase Enzyme

The cellulase enzyme achieved the highest yield, with eight fungal species demonstrating its production, as evidenced by the formation of a yellow halo around the fungal colony resulting from the breakdown of complex carbohydrates into simple sugars.

Amylase Enzyme

Amylase enzyme ranked second, as seven of the examined fungal species had the capacity to degrade starch and produce amylase by creating a yellow halo surrounding the colony, signifying the degradation of starch into simple sugars, while simultaneously coloring the remainder of the medium purple owing to the interaction of starch with iodine.

Lipase Enzyme

The lipase enzyme ranked third, with three fungal species demonstrating remarkable effectiveness in secretion, with no significant differences between them ($P < 0.01$). This was indicated by a clear halo surrounding the fungal colony, resulting from the development of a white precipitate or crystals.

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Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

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