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IDENTIFICATION OF A LIPOLYTIC *Trichoderma* sp. AND CHARACTERIZATION OF ITS EXTRACELLULAR LIPASE

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INTRODUCTION

What are lipases?

- Lipases are a significant group of biocatalysts, hydrolyzing carboxylic ester bonds to release carboxylic acids and alcohols (Daiha *et al.*, 2015)

- Lipases catalyze other industrially demanding reactions such as
  - ✓ Esterification
  - ✓ Transesterification
  - ✓ Acidolysis (Mehta *et al.*, 2017)

- Most of the lipases get activated by a water-lipid interface

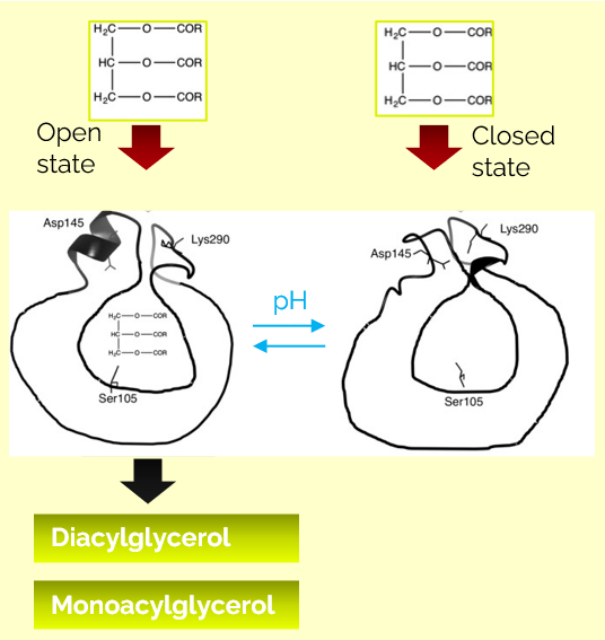


Figure 1: Diagrammatic representation of the overall mechanism of lipase catalysis

Significance of fungal lipases in industries

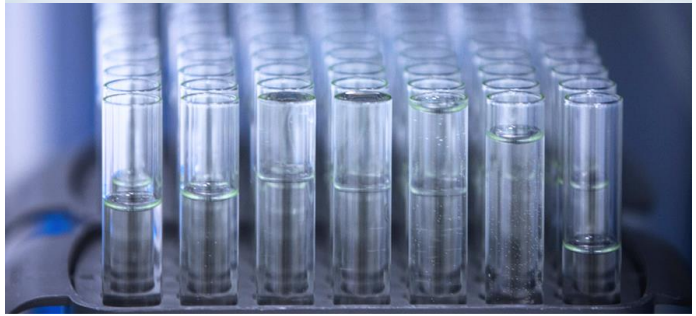
- ✓ **Low cost of production**
- ✓ **Availability**
- ✓ **Ease in genetic manipulation** (Mehta *et al.*, 2017)
- ✓ **Ability to tolerate polar solvents**
- ✓ **Thermostability**
- ✓ **Stability at acidic pH** (Liu *et al.*, 2015)

RESEARCH OBJECTIVES

- ✓ To identify the lipolytic fungus up to species-level

- ✓ To optimize the growth medium for an enhanced lipase production

- ✓ To characterize lipase activity to determine optimized conditions



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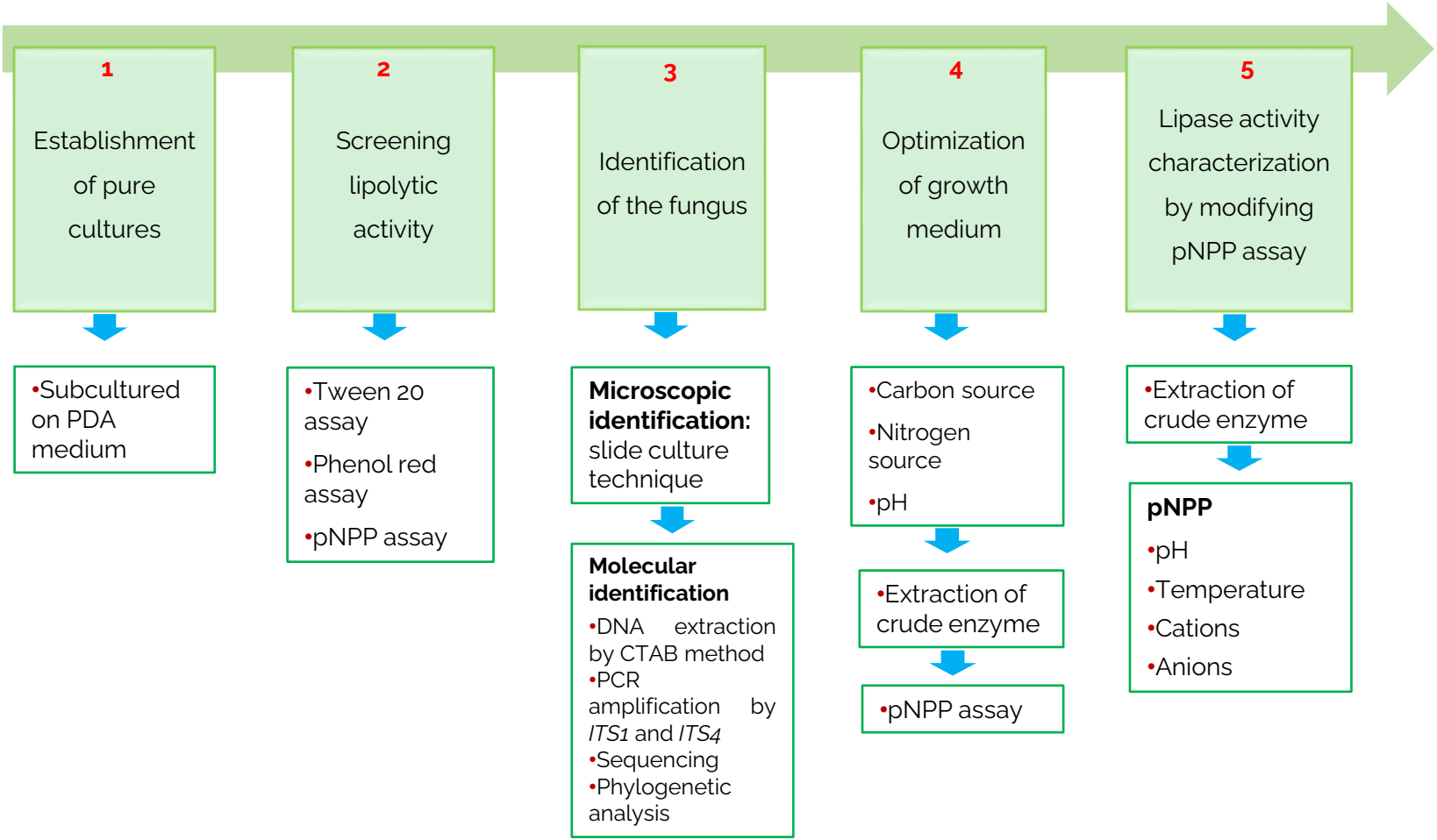
METHODOLOGY


1
Establishment of pure cultures
•Subcultured on PDA medium
2
Screening lipolytic activity
•Tween 20 assay
•Phenol red assay
•pNPP assay
3
Identification of the fungus
Microscopic identification: slide culture technique
Molecular identification
•DNA extraction by CTAB method
•PCR amplification by ITS1 and ITS4
•Sequencing
•Phylogenetic analysis
4
Optimization of growth medium
•Carbon source
•Nitrogen source
•pH
•Extraction of crude enzyme
•pNPP assay
5
Lipase activity characterization by modifying pNPP assay
•Extraction of crude enzyme
pNPP
•pH
•Temperature
•Cations
•Anions

Identification of lipolytic activity

Tween 20 plate assay

Medium components:
Tween 20, peptone, NaCl, $CaCl_2$, agar, and distilled water
pH: 5.8

Phenol red plate assay

Medium components:
Phenol red indicator, olive oil, $CaCl_2$, agar, and distilled water
pH: 7.8

↓

Autoclave
↓
Solidification
↓
Inoculation
↓
Incubation for 3 days at room temperature

para-Nitrophenyl Palmitate (pNPP) assay (Gupta *et al.*, 2002)

Solution 1
pNPP
Isopropanol
Triton-X 100

+

Solution 2
Gum Arabic
Tris-HCl buffer (pH 8.0)

↓

Addition of the extracted crude enzyme
↓
15 min incubation at 40 °C
↓
Absorbance at 410 nm

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RESULTS & DISCUSSION

Pure cultures and lipolytic activity

Figure 2: *Trichoderma* colony on PDA

Tween 20 plate assay

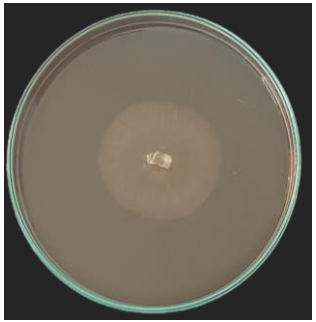


Figure 3: White precipitate in Tween 20 plate assay

Phenol red plate assay

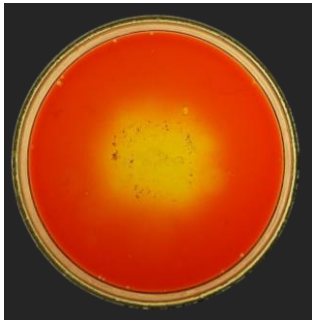


Figure 4: Yellow coloration in Phenol red plate assay

Identification of the fungus

Microscopic identification

- Spores: spherical (S)
- Mycelium: bidirectionally branching (M)

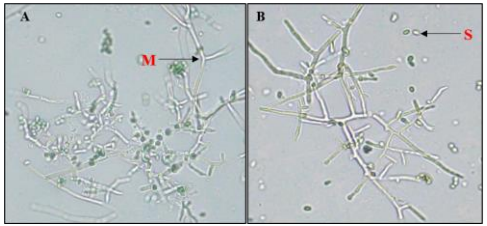


Figure 5: Microscopic image of the fungus stained with lactophenol in cotton blue

Molecular identification

- The amplified DNA fragment resulted in a band of 600 bp in 2 % agarose
- The obtained sequence showed: 100 % query cover
  100 % identity
  0.0 E value

with ***Trichoderma longibrachiatum*** sequence available in online database

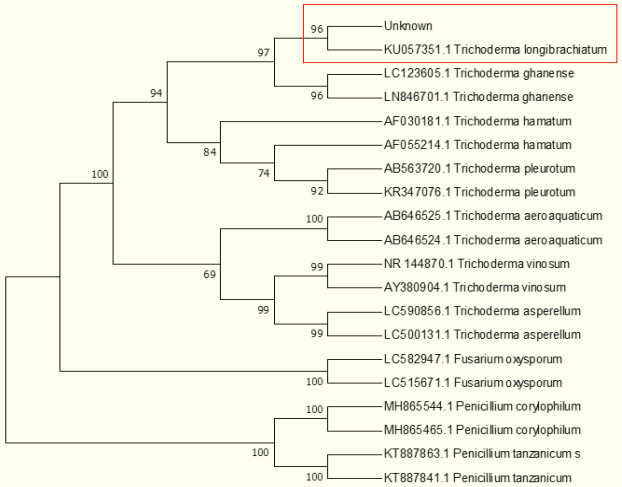


Figure 6: The phylogenetic relationship of the unknown sequence to the selected sequences from the Gen Bank Database.

Optimization of growth medium

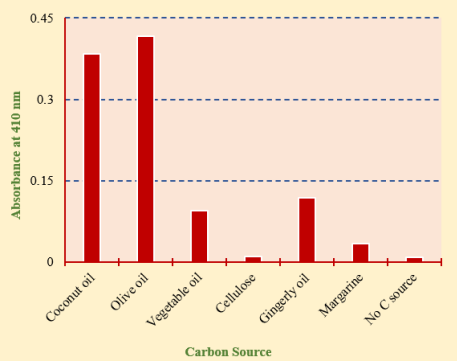


Figure 7: Affect of different carbon sources on lipase secretion from the lipolytic fungus

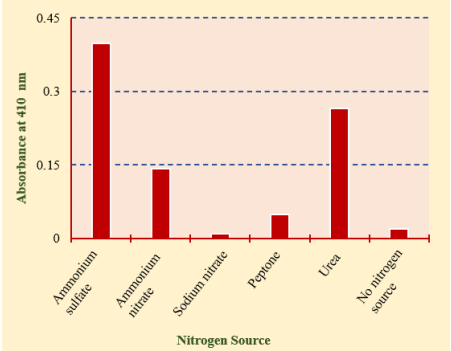


Figure 8: Affect of different nitrogen sources on lipase secretion from the lipolytic fungus

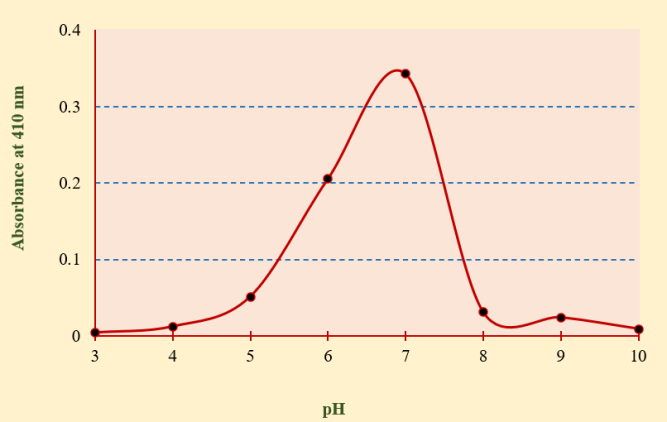
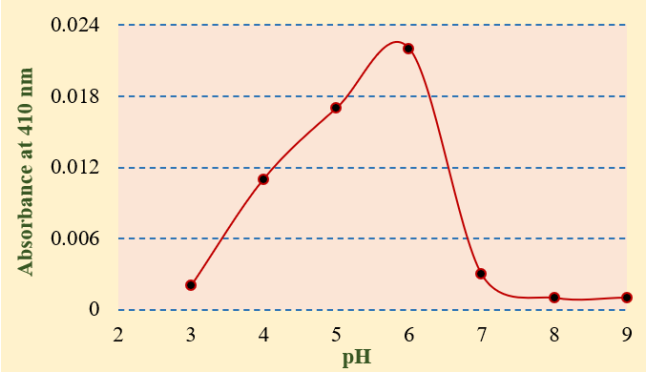


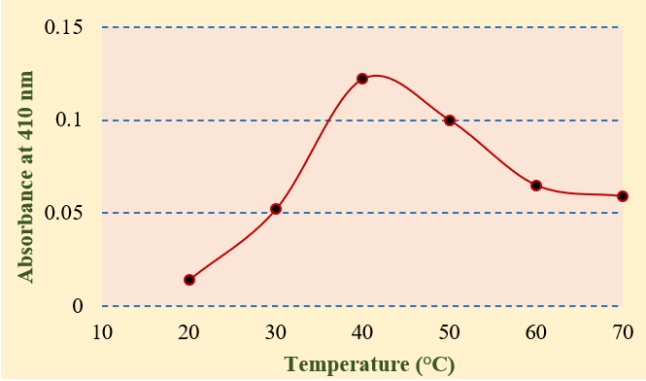
Figure 9: Affect of pH of the growth medium on lipase secretion from the lipolytic fungus

Optimization of lipase activity



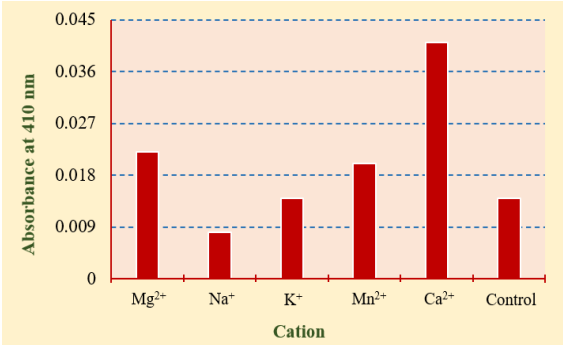
Highest lipase activity at pH 6.0

Figure 10: Influenze of pH on lipase activity



Highest lipase activity at 40 °C

Figure 11: Influenze of temperature on lipase activity



Lipase activity was highest with Ca^{2+} as the cation

Figure 12: Influenze of different cations on lipase activity

Lipase activity was highest with NO_3^- as the anion

Figure 13: Influenze of different anions on lipase activity

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CONCLUSIONS

- ✓ Supported by the microscopic and molecular identification; the lipolytic fungus is *Trichoderma longibrachiatum*

- ✓ The crude lipase secretion from the lipolytic *Trichoderma* species could be increased by media optimization

Maximum lipase secretion achieved with olive oil as the carbon source, ammonium sulfate as the nitrogen source at a pH of 7.0.

- ✓ The crude lipase activity could be enhanced under optimized conditions

The crude enzyme activity highest at a pH of 6.0 and 40 °C. The enzyme activity could be enhanced with Ca^{2+} and NO_3^-.

Future research

- ✓ Further studies are required to test the enzyme's activity at high temperatures and the enzyme's thermostability
- ✓ Characterizing the enzyme activity with frequently used industrially important chemicals can expose more industrially favorable enzyme properties.

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THANK YOU

Any questions?

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