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

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INTRODUCTION

- Lipases are triacylglycerol hydrolases hydrolyzing carboxylic ester bonds to release carboxylic acids and alcohols
- Fungal lipases standing out from other lipases from different sources have gained attention due to their thermostability, stability under extreme pH, and in organic solvents (Mehta *et al*., 2017).
- Fungal lipases are used in a wide range of industries. This demand has encouraged the studies to search for novel fungal lipases. However, media optimization to enhance lipase secretion from the fungus and enzyme characterization are crucial as isolating novel lipolytic fungus.

OBJECTIVES

- To accomplish species-level identification of the lipolytic fungus
- To optimize the fungal growth medium to enhance the lipase secretion
- To characterize crude lipase activity

METHODOLOGY

Establishment of pure fungal cultures › PDA plate › Point inoculation

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Microscopic identification › Slide culture › Lactophenol in cotton blue staining

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Screening lipolytic activity › Tween 20 plate assay › Phenol red plate assay › *para*-Nitrophenyl Palmitate assay

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Molecular identification › DNA extraction › PCR amplification › Sequencing

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Media optimization › C source optimization › N source optimization › pH optimization

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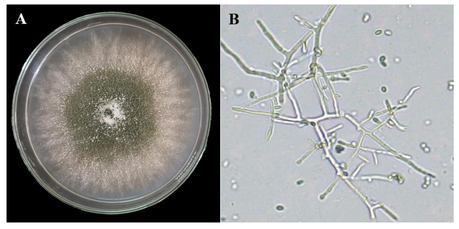
Enzyme activity characterization › Effect of pH › Effect of temperature › Effect of cations › Effect of anions

RESULTS AND DISCUSSION

Pure cultures and microscopic identification

Figure 1: Macroscopic (A) and microscopic (B) observations revealed the lipolytic fungus as a *Trichoderma* sp.



Screening lipolytic activity

Tween 20 assay

A white precipitate due to the calcium salt formed by the fatty acid released by the hydrolysis of Tween 20 was observed around the inoculum.

Phenol red assay

The colour of phenol red indicator changed from red to yellow around the inoculum due to the formation of fatty acids by the lipolytic fungus.

Figure 2: Observation of Tween 20 assay.

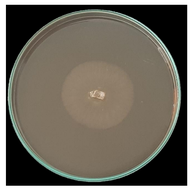
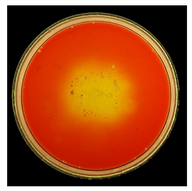


Figure 3: Observation of phenol red assay.



Quantitative screening of lipase activity using *para*-nitrophenyl palmitate assay indicated the highest lipase secretion on the third day of incubation.

Molecular Identification of the fungus

The DNA fragment amplified using *ITS1* and *ITS4* primers resulted in a band of 600 bp in 2 % agarose gel. The obtained nucleotide sequence showed 98 % query cover, 100 % identity, and 0.0 E value with *Trichoderma longibrachiatum* sequence available in Gen Bank Database.

Media optimization

Figure 4 : Carbon source optimization.

Olive oil exhibited the highest lipase secretion from the *Trichoderma* sp.

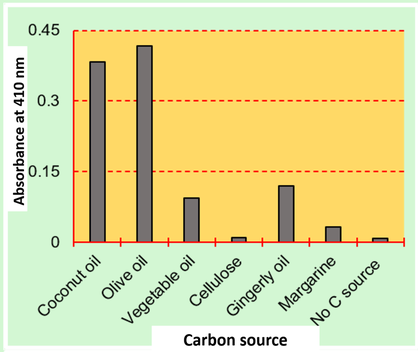


Figure 5: Nitrogen source optimization.

Ammonium sulfate as the nitrogen source reported the highest lipase secretion.

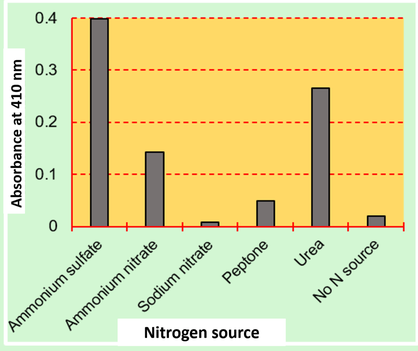
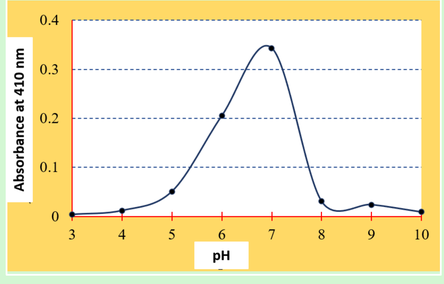


Figure 6: pH optimization.

The lipase secretion from the lipolytic fungus was highest at pH 7.0 Alkaline pH inhibited lipase production.



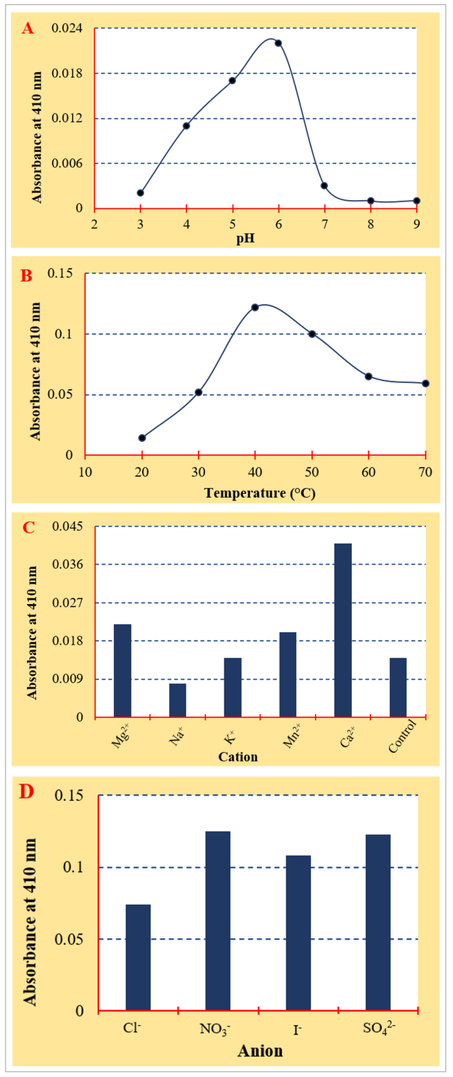
Enzyme activity characterization

Figure 7 A: Effect of pH. Changing pH affects the charges on amino acids forming the enzyme, thereby affecting the active site's shape. For the studied lipase, the activity was highest at pH 6.0, while the enzyme activity was almost negligible at alkaline pH.

Figure 7 B: Effect of temperature. The crude lipase activity was highest at 40 °C. However, a considerable lipase activity was observed at high temperatures.

Figure 7 C: Effect of different cations. Lipases are metalloenzymes requiring metal ions in their active site for catalytic activity. Ca^{2+} enhanced enzyme activity for the tested crude lipase.

Figure 7 D: Effect of different anions. Crude enzyme activity was highest with NO_3^-.



CONCLUSIONS

- The molecular identification indicated the lipolytic fungus as *Trichoderma longibrachiatum*.
- The lipase secretion from the lipolytic *Trichoderma* species could be increased by olive oil and ammonium sulfate, at pH 7.0.
- Crude lipase activity could be enhanced at pH 6.0 and 40 °C, and with Ca^{2+} and NO_3^-.

REFERENCE

Mehta, A., Bodh, U. and Gupta, R. (2017). Fungal lipases: a review. *Journal of Biotech Research* **8**(1)**:** 58-77.