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

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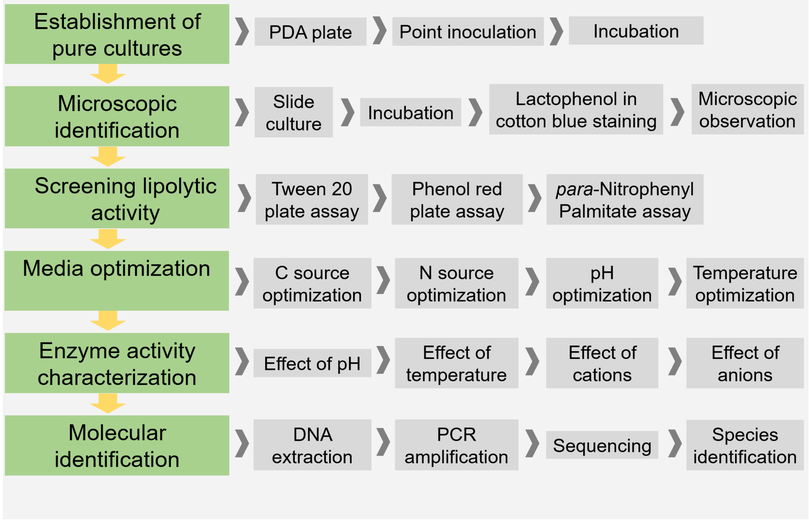
ABSTRACT

Fungal lipases are a significant group of biocatalysts abundantly used in a wide range of industries. The demand for lipases has kept researchers exploring new lipolytic fungi and characterizing their extracellular lipase. Therefore, the current research has been undertaken to characterize the crude enzyme extracted from a lipolytic *Trichoderma* species and to accomplish species-level identification of the fungus. The optimization of the growth medium revealed that olive oil and ammonium sulfate at a pH of 7.0 could enhance lipase production. The crude enzyme activity was significant with Ca^{2+} and NO_3^- at a pH of 6.0 and 40 °C. DNA sequencing revealed the fungus as *Trichoderma longibrachiatum*. The results uncovered that this lipolytic fungus could be improved for industrial applications.

INTRODUCTION

Lipases are triacylglycerol acyl hydrolases hydrolyzing carboxylic ester bonds to release carboxylic acids and alcohols. Lipases are a versatile group of enzymes due to their chemo selectivity, stereoselectivity, availability, independence on cofactors, and activity in organic solvents. Lipases are a preferred type of catalyst in a wide range of industries (Daiha *et al*., 2015). Fungal lipases standing out from other lipases from different sources have gained attention due to their thermostability, stability under extreme pH, and stability in organic solvents (Mehta *et al*., 2017). The potential for a wide field of applications has encouraged the studies to search for novel fungal lipases. Media optimization to enhance lipase secretion from the fungus and optimization of enzyme activity are crucial as isolating novel lipolytic fungus. Characterization studies on several *Trichoderma* lipases have revealed several significant properties of the enzymes. The current study has been undertaken to optimize the growth medium of a previously isolated lipolytic *Trichoderma* species and optimize the conditions for enhanced lipase activity. Moreover, this research was conducted to identify the species of lipolytic *Trichoderma* by molecular identification techniques.

METHOD



RESULTS AND DISCUSSION

Pure cultures and microscopic identification

The morphology of the pure fungus colony on PDA and microscopic observations revealed the lipolytic fungus as a *Trichoderma* sp.

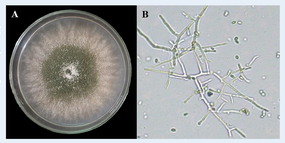


Figure 1: Macroscopic and microscopic observations of the fungus. A; Colony morphology on PDA. B; Microscopic observation by slide culture technique.

Screening lipolytic activity

In the Tween 20 plate assay, the formation of a precipitate of white due to the calcium salt formed by the fatty acid released by the hydrolysis of Tween 20 was observed around the inoculum. The color of phenol red indicator changed from red to yellow around the inoculum in the phenol red plate assay due to the formation of fatty acids by the lipolytic fungus.

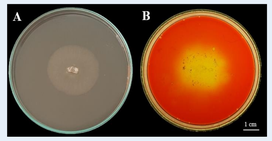


Figure 2: Primary screening of lipolytic fungus by plate assays. A; Observation of Tween 20 plate. B; Observation of phenol red assay.

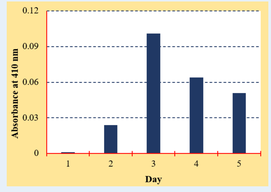


Figure 3: Quantitative screening of lipase activity using *para*-nitrophenyl palmitate assay. A 3 day incubation period showed the highest lipase production.

MEDIA OPTIMIZATION

Olive oil as the carbon source exhibited the highest lipase secretion from the *Trichoderma* sp. The lowest lipase secretion in the absence of a carbon source revealed the need for a carbon source for lipase production by the fungus. Ammonium sulfate as the nitrogen source reported the highest lipase secretion, while sodium nitrate showed an inhibitory effect.

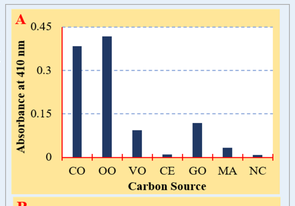


Figure 4 A: Carbon source optimization. The x-axis indicates different carbon sources; CO: Coconut oil, OO: Olive oil, VO: Vegetable oil, CE: Cellulose, GO: Gingerly oil, MA: Margarine, and NC: No carbon source.

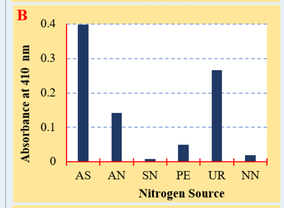


Figure 4 B: Nitrogen source optimization. The x-axis indicates different nitrogen sources; AS: Ammonium sulfate, AN: Ammonium nitrate, SN: Sodium nitrate, PE: Peptone, UR: Urea, and NN: No nitrogen source.

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

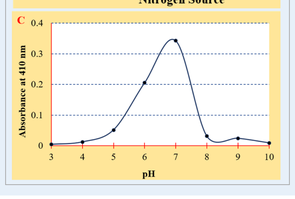


Figure 4 C: pH optimization. The x-axis indicates the tested pH range, while the y-axis indicates the absorbance readings at 410 nm.

ENZYME ACTIVITY CHARACTERIZATION

Changing the pH, temperature, cations, and anions during the crude lipase activity uncovered the effect of those factors on lipase activity.

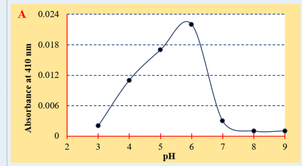


Figure 5 A: Effect of pH. Changing pH affects the charges on amino acids forming the enzyme, thereby affecting the active site's shape. For the studied lipase, the activity was highest at a pH of 6.0, while the enzyme activity was almost negligible at alkaline pH.

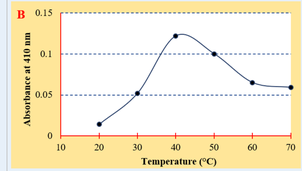


Figure 5 B: Effect of temperature. The crude lipase activity was highest at a temperature of 40 °C. High temperatures lead to protein denaturation, disrupting the shape of the active site and reduces enzyme activity. However, for the tested crude lipase, considerable enzyme activity was observed at high temperatures.

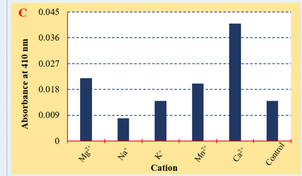


Figure 5 C: Effect of different cations. Lipases are metalloenzymes requiring metal ions in their active site for catalytic activity. Ca^{2+} enhanced enzyme activity for the tested crude lipase, while Na^+ showed an inhibitory effect compared to the control without any cation incorporation.

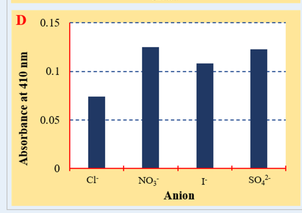


Figure 5 D: Effect of different anions. Crude enzyme activity was highest with NO_3^-. The enzyme activity was also significant with SO_4^{2-} and I^-. Cl^- resulted in the lowest lipase activity.

MOLECULAR IDENTIFICATION

Distinguishing different species of *Trichoderma* by observing morphological characteristics alone is difficult and often leads to misidentifications of species. The DNA fragment amplified using *ITS1* and *ITS4* primers resulted in a band of 600 bp in 2 % agarose gel. The obtained sequence showed 98 % query cover, 100 % identity, and 0.0 E value with *Trichoderma longibrachiatum* sequence available in online databases.

CONCLUSIONS

The current study results revealed that the crude lipase secretion from the lipolytic *Trichoderma* species could be increased by olive and ammonium sulfate, as the carbon and nitrogen sources, respectively, at a pH of 7.0. The enzyme activity characterization studies revealed that the crude lipase activity could be enhanced at a pH of 6.0 and a temperature of 40 °C. Moreover, the enzyme activity could be enhanced using Ca^{2+} and NO_3^-. The molecular identification indicated the lipolytic fungus as *Trichoderma longibrachiatum*.

REFERENCES

Daiha, K., Angeli, R., de Oliveira, S. D., & Almeida, R. V. (2015). Are lipases still important biocatalysts? A study of scientific publications and patents for technological forecasting. *PloS one* **10**(6): e0131624. DOI: https://doi.org/10.1371/journal.

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