Production and Optimization of Lipase Enzyme Through Solid State Fermentation, by Brown Molds, Using Cotton Seed Cake.

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ABSTRACT

Nowadays, enzymes have replaced expensive chemical usage in reactions carried out in industries to produce specific products. There are many enzymes having industrial importance, lipase is one of them. So, in this study, the potential of lipase from fungal strains, isolated from soil, is exploited. In the current work, lipase was produced through solid state fermentation by brown molds, isolated from soil. The production parameters including type of substrate, concentration of substrate, temperature, pH, and incubation time, were optimized. The enzyme activity for each parameter was found through turbidimetric assay. The highest enzyme activity was found in the culture medium containing 10g cotton seed cake as substrate and tween 20 as emulsifier, having pH 6, and incubated at 35°C for 5 days.

Keywords: Cotton seed cake, Fungal lipase, lipase production, Optimization.

INTRODUCTION

Lipase is one of the important industrial enzymes.[1] and is produced by many living beings but the most preferred source of lipase is microorganisms because of their low production cost and higher yield[2]. Among microorganisms, fungus is considered to be a good option, facilitating the extraction and purification steps. Lipase (EC 3.1.1.3) is a triglyceride acyl ester hydrolase which hydrolyzes the triglycerides into fatty acid and glycerol over an oil-water interface. Other than this, lipase also catalyzes the reactions including acidolysis, aminolysis, esterification and transesterification[3]. They have a lot of applications in many industries which includes detergent and biodiesel production, and are also utilized in textile industry, food industry and pharmaceutical industry[4]. So,to fulfill the demand of the enzyme in the market, there has been a continuous search for its economical production. For this, agricultural waste products and media optimization are two best approaches[5]. So, in the present research, one of the cheap rawmaterial, cotton seed cake, that no one has used till now for fungal lipase production, is used and the enzyme is produced through solid state fermentation, optimizing different parameters

OBJECTIVES

- Identification of lipase producing fungal strain.
- Production of extracellular lipase enzyme by isolated fungal strain.
- Efficient production of lipase enzyme using cheap raw material.
- Optimization of production parameters to have greater yield of lipase enzyme.

METHODOLOGY

Sample collection and isolation of fungal strains:

Different soil samples were collected from different sites. Each sample was serially diluted, inoculated onto the PDA plate, and the resulting growth was then isolated, using the same media.

Screening and identification of lipase producing strains:

The fungal isolates were screened against nutrient agar, supplemented with tween 20 (1.8%) as substrate. Two strains, brown mold and orange mold, which showed greater lipolytic activity were selected. The selected molds were identified as Aspergillus, after microscopy, as shown in figure **1**,

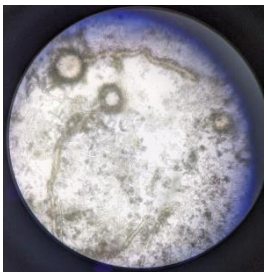

Figure 1. Brown molds under microscope.

Solid state fermentation and lipase recovery:

For lipase production, 2% inoculum was added (brown mold and orange mold) into the autoclaved lipase production media containing 0.5% glucose, .5% peptone, 0.5% yeast extract, 0.05% MgSO4.7H2O, 0.3% NaCl and 5% Olive oil in distilled water. The flasks were then incubated at 37°C for 5 days.

After fermentation, the crude enzyme was recovered through filtration.

Enzyme activity:

Plate assay:

The crude enzyme was screened for lipase activity through plate assay.

Turbidimetric assay:

Unit calculation was done by turbidimetric assay taking absorbance at 500nm.

Optimization:

The product parameters were optimized with Solid state fermentation.

Effect of Substrate on Lipase production:

Different substrates including rice husk, cotton seed cake, mustard oil, wheat crush, and blackpea seed coat were investigated for higher yield of lipase.

Effect of Substrate concentration on Lipase production:

The effect of Substrate concentration was determined, taking different concentrations of substrate i.e 2.5g, 5g, 10g, 20g having lipase production media in ratio 1:1.

Effect of Temperature on Lipase production:

To determine the optimum temperature, five flasks, each containing fermentation media, inoculated with spores suspension, were incubated at different temperatures 25°C, 30°C, 35°C, 40°C and 45°C respectively.

Effect of PH on Lipase production:

To determine the best pH, fermentation media was prepared in five flasks, having pH 3, 4, 5, 6,7, 8 respectively and were incubated at 37°C for 5 days.

Effect of emulsifier on Lipase production:

Three emulsifiers were selected namely triton X-100, tween 20, and tween 80, each was taken1% in the fermentation media.

Effect of incubation time on Lipase production:

Five flasks containing fermentation media, inoculated with spore suspension were incubated for different time intervals, 3 days, 5 days, 7 days, and 9 days, each at 37°C.

RESULTS

The Brown molds were identified as the most proficient lipase-producing strain among the isolated strains. The highest lipase production was observed when utilizing cotton seed cake as substrate, yielding an enzymatic activity of 3452U/ml. The best concentration for substrate was shown at 10g (3190U/ml). The most favorable conditions were found to be at temperature 35°C (2990U/ml), pH 6 (3145U/ml), and Tween 20 as an emulsifier (3000U/ml). Furthermore, the ideal incubation time observed was 5 days (3244U/ml).

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