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Production of lovastatin by *Aspergillus terreus* on groundnut shell through solid-state fermentation

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ABSTRACT

The main purpose of this study was to screen the fungal culture for the production of lovastatin on Groundnut shell wastes in dextrose and urea based medium Quantitative analysis of lovastatin was performed by UV spectroscopy method. The total lovastatin yield achieved was 0.052 mg/ml after 10 days of fermentation conducted at 30ºC temperature with an initial pH of 5.5. This experimental result indicates that optimized culture conditions were used for higher yields of lovastatin on groundnut shell wastes.

KEYWORDS

Aspergillus terreus;
Lovastatin;
Groundnut shell;
Solid state;
Fermentation.

INTRODUCTION

Lovastatin, a potent drug for lowering blood cholesterol, which acts by competitively inhibiting the enzyme 3-hydroxyl-3-methylglutaryl coenzyme A reductase (HMG-CoA), which catalyzes the rate limiting step of cholesterol biosynthesis[1]. It has also been reported as a potential therapeutic agent for suppressing tumor growth through the inhibition of nonsterol isoprenoid synthesis[2]. Lovastatin is produced by various filamentous fungi such as *Aspergillus terreus*, *Penicillium citrinum* and *Monascus rubber* (Xu et al. 2005). Initially, several agro-industrial lignocellulosic waste substrates such as wheat bran, corn hull, rice husk, sugarcane bagasse, orange peel, orange pulp, cotton seed oil cake and groundnut oil were screened for the production of lovastatin. Among several studies conducted so far, wheat bran was reported as supporting the highest production of lovastatin. Groundnut shell is an important oil seed crop of India. The pod or dry pericarp contains about 25-40 percent shell. In the present study, the potentiality of groundnut shell to be used as substrate for lovastatin production by fungal strain *Aspergillus terreus*.

MATERIALS AND METHODS

Microorganism

Aspergillus terreus was procured from the cultures being maintained at the department of Microbiology Sri

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Venkateswara University, Tirupati, India.

Substrate

Groundnut shell wastes collected from local household were powdered and soaked in water for about 12 hours. Excess water was drained off and the powder was dried at 70ºC in oven for 24 hours. The powder was then sieved through 2mm mesh and kept at room temperature for further studies.

Inoculum preparation

Aspergillus terreus was grown on potato-dextrose agar slants at maintained at 30°C for 7 days until complete sporulation. Five milliliters sterile aqueous solution of 2% Tween -20 was added to the slant and the spores were scraped and used as inoculum. The spore concentration was determined as 10^7 per ml of inoculum by using Neubar chamber.

Solid state fermentation for production of lovastatin

Groundnut shell (10 grams) was moistened with Mandel and Reese medium (Dextrose, 100; Peptone, 1.0; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; NH_2-CO-NH_2, 0.3; $MgSO_4.7H_2O$,0.3; $CaCl_2$, 0.3; $FeSO_4.7H_2O$, 0.005; $MnSO_4$.H2O, 0.0016; $ZnCl_2$, 0.0017; Distilled water, 1000 ml; pH 6.5) to initial moisture content (50%) and autoclaved in 250 ml flask at 121ºC for 1 hour. After cooling, flasks were inoculated with 5ml of 1×10^7 fungal spores per milliliter inoculum. The contents in the flask were mixed thoroughly to ensure uniform distribution of the inoculum and flasks were incubated at 30ºC for 10 days. The lovastatin was extracted in

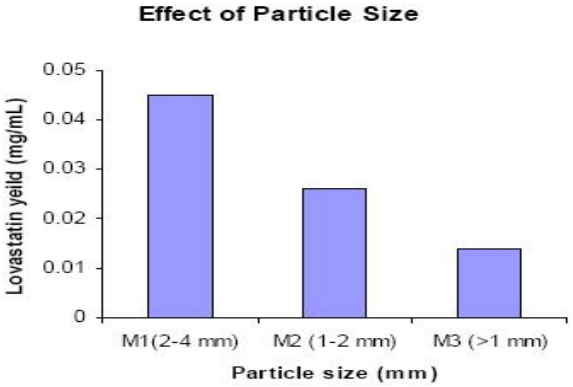

Figure 1 : Effect of particle size on lovastatin production *by Aspergillus terreus*. *Values represented in fig are mean of duplicates

methanol as a organic solvent.

Extraction of lovastatin with organic solvent

Both hydroxy acid and lactone (drug form) forms of lovastatin produced during fermentation were extracted in Methanol in two stages. In the first step, 100 mL of organic solvent (methanol) was mixed with 2.5 g of solid matrix and mixed vigorously for 5 min, followed by incubation in a rotary shaker at 30°C, 200 rpm for 2 h. In the second step, the extract was filtered to separate the biomass, and centrifuged at 10,000 rpm in ultra centrifuge (Remi) for 10 min for separation of fungal spores from the extract. Clear extract obtained was stored in glass bottles at 4°C until analysis.

Estimation of lovastatin

Lovastatin was estimated by adjusting the pH to 5.5 with diluted phosphoric acid (H_3PO_4). The extract was diluted to 5 times with methanol and absorbance was read at 238 nm in UV Spectrophotometer (Elico) by the method of Chakravarti and Sahai[5]. The values were compared with standard calibration graph of lovastatin (Reddys lab Hyderabad, India).

RESULTS

The aim of this study was to find the potentiality of lovastatin production by *Aspergillus terrues* through solid state fermentation on groundnut shell wastes with different particles sizes were tested and listed (Figure 1) various particle sizes used in the present study viz., M1 (2.0 and 4.0 mm), M2 (1.0 and 2.0mm) and M3 (less than 1.0mm).maintained at 30ºC incubation temperature.

The groundnut particle size at 2-4 mm was more suitable for lovastatin production (Figure 1).

Effect of temperature

Keeping particle size constant (2 mm), the fermentation process was carried out at five different temperatures of incubation to find optimal incubation temperature for maximum production of lovastatin. The yields of lovastatin at respective temperatures are shown in figure 2.

It was found that at 30ºC, optimum concentration of lovastatin was obtained (0.045 mg/ml) after 10 days of incubation. Fluctuation in temperature more or less

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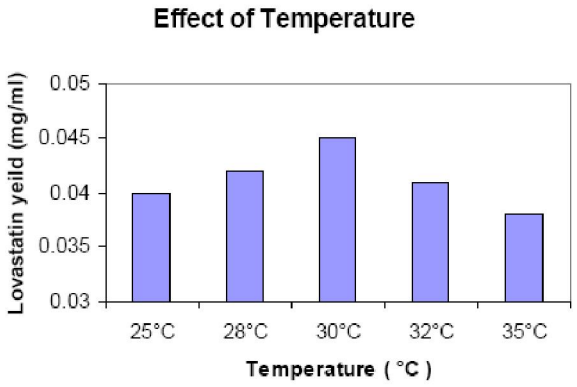

Figure 2 : Effect of temperature on lovastatin production by *A.Terreus*. *Values represented in fig are mean of duplicates

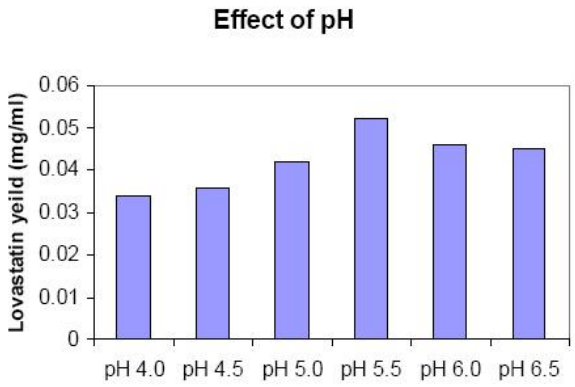

Figure 3 : Effect of pH on lovastatin production by *Aspergillus terreus*. *Values represented in the fig are mean of duplicates

was reflected on fermentation production. This confirms that the design of fermentation medium is critical; especially when the products are secondary metabolites (Atalla et al., 1999).

Effect of pH

The effect of varying pH on the yield of lovastatin was examined keeping particle size and temperature at optimum level. The results obtained were as in the figure 3.

It was found that maximum lovastatin production was 0.052 mg/ml after 10 days of fermentation when production medium was at initial pH of 5.5. It was also observed that decrease or increase in pH of the fermentation solution was accompanied by decrease in production. The results slightly differed with those of Alberts et al.[3] and Kysilka[8] who adjusted the pH of the fermentation medium at 7.4 to produce lovastatin by *A. terreus* on rice medium.

DISCUSSION

In the present study, the potentiality of groundnut shell waste to be used as solid substrate for the fermentation production of lovastatin has been tested. Methanol was used for the extraction of both hydroxy acid and lactone (drug form) forms of lovastatin. Lactone form (drug form) of lovastatin was estimated in this study by UV spectroscopy method with a standard reference. The maximum yield of 0.052 mg/ml lovastatin was obtained at an initial medium pH of 5.5, incubation temperature of 30ºC after a fermentation period of 10 days with particle size 2 mm.

The results obtained in this study are highly encouraging and further studies can be conducted to optimize other parameters such as carbon and nitrogen sources and any other supplements for enhanced production of lovastatin on groundnut shell wastes.

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