Production and Optimization of Mevastatin using *Penicillium citrinum* NCIM 768

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**Abstract**

A modified solid-state fermentation was used to produce mevastatin by *Penicillium citrinum* NCIM 768 recycling wheat bran as carrier. The following optimum conditions of physicochemical parameters of temperature 27°C, pH 4 with a relative humidity of 60% and at an inoculum volume of 2.5 ml, resulted in mevastatin yield of 68.7 mg L⁻¹ in fermentation broth. Among various nitrogen and carbon sources, addition of sodium nitrate and glucose improved mevastatin production.

**Keywords:** Mevastatin; Anticholesterol; *Penicillium citrinum*; Solid fermentation; Secondary metabolite

**Introduction**

Mevastatin, also known as compactin or ML-236B, is a member of the class of statins belonging to the polypeptide group and it is a hypocholesteremic agent that has been an attractive molecule over the last few decades, for its anticholesterol activity [1]. It is soluble in ethanol and dimethyl sulfoxide. Mevastatin was highly effective in lowering the total and low-density lipoprotein (LDL) cholesterol in both experimental animals and patients with primary hypercholesterolemia [2].

The hypocholesterolemic effect of statins lies in the reduction of the very low-density lipoproteins [VLDL] and LDL involved in the translocation of cholesterol [3], and in the increased level of high-density lipoproteins [HDL] [4], with a subsequent reduction of the LDL to HDL-cholesterol ratio, which is the best predictor of atherogenic risk [5].

Mevastatin, a secondary metabolite and its derivative is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase [6], which is a regulatory enzyme for cholesterol biosynthesis. This is a rate-limiting enzyme in cholesterol synthesis in humans and act as a useful drug against atherosclerosis. Pravastatin can also be obtained by the biotransformation of mevastatin by *Streptomycetes carboxophilus* [7].

Mevastatin has been produced in submerged fungal fermentation of glucose medium using *Penicillium citrinum*, NCIM 768 in the recent years, there has been increasing interest in the use of solid-state fermentation [SSF]. In the current study, we used wheat bran as a carrier in solid state fermentation for the production of mevastatin [8].

**Materials and Methods**

**Procurement of microorganism**

*Penicillium citrinum* NCIM 768 was procured from NCL, Pune, India. The culture was maintained in Potato Dextrose Agar (PDA) slants.

**Inoculum preparation**

The water soluble extract of soybean meal (40g soybean was kept in 1000 ml of distilled water at 4°C under stirring conditions for 6 days) was prepared. pH of the medium was adjusted to 6.3 and sterilized in the autoclave at 121°C for 20 minutes at 15 Psi. After sterilization the medium was inoculated with spore suspension at 1% v/v and incubated at 28°C, 220 rpm in an orbital shaker for 26 h.

**Solid state fermentation (SSF)**

Solid state growth experiments were conducted in 250 ml Erlenmeyer flasks containing 10g of wheat bran moistened with 1:1 mineral salt solution [g L⁻¹; K₂HPO₄; NaCl; MgSO₄.7H₂O; FeSO₄] [9], Flasks were sterilized by autoclaving for 15 min at 121°C. After sterilization, the flasks were cooled and inoculated with 5 ml spore suspension (10⁻⁷-10⁻⁸ Spores mL⁻¹) and incubated at 37°C for 144 hrs [10,11], Samples were taken for analysis of mevastatin production for each 24 h. All the experiments were carried out in triplicates and the results were expressed in mean with standard deviations.

**Optimization of pH, temperature and inoculum volume**

Optimization of various process parameters were carried out to enhance production of mevastatin under solid state growth conditions. The growth studies were carried out considering different parameters such as temperature, pH of the medium and the volume of inoculum. Experiments were performed using wheat bran as a substrate with different pH ranges from 2-6 and it was kept at 60% relative humidity [9], Likewise the solid substrate was kept at different temperature ranged from 27°C, 37°C, 45°C, 50°C and 60°C. Fermentation was carried out with different inoculum from 1.5 to 3.5 ml to get optimum inoculum volume for maximum production of mevastatin.

**Supplementation with different carbon and nitrogen sources**

Different carbon sources such as glucose, fructose, sucrose, maltose was used. Optimization of the fermentation broth was conducted by recycling wheat bran as carrier. The following optimum conditions of physicochemical parameters of temperature 27°C, pH 4 with a relative humidity of 60% and at an inoculum volume of 2.5 ml, resulted in mevastatin yield of 68.7 mg L⁻¹ in fermentation broth. Among various nitrogen and carbon sources, addition of sodium nitrate and glucose improved mevastatin production.
and starch and nitrogen sources such as urea, peptone, yeast extract, sodium nitrate and ammonium sulphate on mevastatin production were also studied. These sources were supplemented with wheat bran concentration of 1% (w/w) on dry basis, to see their effects on mevastatin production. The final moisture content 60% was maintained throughout the study.

**Loss of organic matter**

Material was dried before and after fermentation up to constant weight. Loss of Organic Matter (LOM) was calculated as the weight difference and expressed as a percentage of the initial dry weight of the samples.

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\text{LOM} = \left( \frac{W_i - W_f}{W_i} \right) \times 100 \%
\]

Where \( W_i \) is the initial dry weight of solid material before fermentation and \( W_f \) is the final dry weight of solid material after fermentation.

It was assumed that the loss of organic matter is due to CO\(_2\) formation by fungal metabolic activities during the course of fermentation. Since there is no direct method for biomass estimation in SSF, LOM was used to express biomass and metabolic activity in an indirect way.

**Estimation of mevastatin**

At the end of fermentation, the biomass was treated with 50 ml of distilled water and agitated thoroughly on orbital shaker for 30 minutes. The whole contents were filtered through whatman No.1 filter paper and absorbance was read to quantify the mevastatin based on the standard chart plotted using standard sigma grade mevastatin (\( \lambda_{\text{max}} = 238 \text{ nm} \)) using UV spectrophotometer [12].

**Results and Discussion**

A detailed study was carried out for mevastatin production by *Penicillium citrinum* NCIM 768 in solid state fermentation using wheat bran as a substrate. Solid substrate other than wheat bran causes agglomeration, which in turn affects mixing, results in the heat accumulation, improper heat transfer and causes reduction in the mevastatin production [13]. Since, low cost solid substrates are used solid-state fermentation has been preferred over submerged fermentation. And different combinations of solid substrates were studied to determine if these could be used to formulate a commercially attractive substrate. Only the wheat bran – barley combination has served reasonably well for the mevastatin production.

**Selection of the carrier**

Wheat bran and Barley were used as carriers for the production of mevastatin by solid state fermentation. Among them, wheat bran enhanced the maximum yield of mevastatin production and it was found to be 68.7 mg L\(^{-1}\) at 60% humidity & 27°C temperature for 6 days of fermentation. The Loss of Organic Matter with 60% moisture was obtained as 30.29%. A Loss of Organic matte above 30% indicates that the fungal growth has almost ceased. This in turn would terminate the synthesis of mevastatin.

The selection of suitable moisture content has been considered as one of the important parameters in the optimization of solid state fermentation. Moisture content has been reported to influence the physical properties of the substrates. High moisture content in the substrate would reduce the porosity of the bed, resulting in poor oxygen transfer into the compact bed. On the other hand, low moisture content is also detrimental, as they convert the vegetative cells into spores; thereby both the biomass and product yields are affected.

**Effect of temperature on mevastatin production**

The solid-state fermentation process at 60% of relative humidity was carried out at different temperature of incubation to get optimal temperature for maximum production of mevastatin viz 27°C, 37°C, 47°C, 57°C, and 67°C. The results are presented in Table 1. The yield of mevastatin was found to be maximum at 27°C, which was 68.7 mg L\(^{-1}\) for 6 days of fermentation. The loss of organic matter with 60% moisture was obtained as 30.29%.

![Figure 1: Effect of Temperature on Mevastatin Production.](image)

**Plate 1:** Initial growth of *Penicillium citrinum* in Rose Bengal agar.

**Plate 2:** Back view of *Penicillium citrinum*.

**Plate 3:** Aerial view of *Penicillium citrinum*.  

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45°C, 50°C & 60°C with wheat bran. It was found that 27°C as the optimum temperature yielding mevastatin of 49.6 mg L⁻¹ after 6 days of fermentation (Figure 1). Plates [1,2,4,10,13], show the culture of *Penicillium citrinum* growth on agar plates (Plates 1 to 3), its microscopic view (Plate 4) and growth in solid state medium (Plate 5).

**Effect of pH on the production medium**

By maintaining other conditions at optimum level (i.e., temperature of incubation at 27°C, relative humidity at 60%), maximum mevastatin production of 81.7 mg L⁻¹ was obtained at initial pH of 4 (Figure 2).

**Effect of seed volume (Inoculum Volume)**

Fermentation was carried out with different inoculum from 1.5 to 3.5 ml to get optimum inoculum volume for maximum production of mevastatin with wheat bran, temperature at 27°C and at relative humidity of 60%. It was found that at inoculum volume of 2.5 ml produced mevastatin of 96.3 mg L⁻¹ after 6 days of incubation (Figure 3).

The higher production of mevastatin is obtained at inoculum volume of 2.5 ml. It was observed that the increase in the mevastatin production was attributed by the drop in biomass growth. Acidic environment at pH 4 with 60% moisture favored the transfer of metal ions and other vital nutrients into fungal cells required for metabolic reactions of the organisms resulting in better product formation. Mevastatin production was optimum at 144 h of incubation at 27°C and it found to be 68.7 mg L⁻¹. It was also observed that there was no further increase in the mevastatin production after 144 h of incubation. This observation prompted us to fix 144 h as the optimal incubation time for the harvest of the product from the solid substrate.

**Effect of carbon source**

Fermentation was carried out with different carbon sources (1%) such as glucose, fructose, sucrose, maltose and starch to get the maximum production of mevastatin with wheat bran, while keeping other conditions at optimum level. It was found that among the carbon source, glucose enhanced the yield of mevastatin production as 73.1 mg L⁻¹ (Figure 4).
Effect of nitrogen source

Fermentation was carried out with different nitrogen sources (1%) such as urea, peptone, yeast extract, sodium nitrate and ammonium sulphate to get the maximum production of mevastatin with wheat bran, while keeping other conditions at optimum level. It was found that the nitrogen source, sodium nitrate an inorganic source enhanced the high yield of mevastatin production as 93.6 mg L⁻¹ (Figure 5).

Finally experiments carried out at the optimal conditions obtained, resulted in the mevastatin production of 95 mg L⁻¹. (Figure 6) shows the time course of mevastatin production at the optimal conditions of physicochemical parameters. It can be observed from the graph that the mevastatin production started after 4th day, indicating the onset of the stationary phase, which in turn favored the mevastatin production.

In the present research, addition of different nitrogen additives to wheat bran resulted in better yield. Among them, sodium nitrate enhanced mevastatin production. Likewise, addition of different carbon additives to wheat bran resulted in better yield. Among them, addition of glucose exhibited better mevastatin production [14]. The prediction of loss of organic matter with sufficient moisture is due to CO₂ formation by fungal metabolic activities during the course of fermentation.

When these results are compared with the literature [13], some variations were observed due to the change in the environmental condition and various levels of physical parameters.

References