

ORIGINAL RESEARCH ARTICLE

Lovastatin production by *Aspergillus fischeri* under solid state fermentation from coconut oil cake

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Abstract:

The main aim of the present investigation was to optimize the fermentation parameters that enhance the maximum production of lovastatin by *Aspergillus fischeri* using coconut oil cake as the solid substrate under solid state fermentation. The maximum yield of lovastatin (14.77 mg/g dry substrate) using coconut oil cake as the substrate was achieved with the following optimized process parameters: fermentation time (7 days), initial moisture content (60% v/w), inoculum volume (2ml of five day old culture), initial pH (5.0), incubation temperature (30°C), lactose (1% w/v) and malt extract (1% w/v).

Keywords: Lovastatin, *Aspergillus fischeri*, Coconut oil cake, Fermentation parameters, Optimization

Introduction:

Lovastatin also called as Mevinolin, Monacolin K and Mevacor, a kind of fungal metabolite, serves as one of the competitive inhibitors of enzyme hydroxymethylglutaryl coenzyme A reductase (HMG-CoA), which catalyzes the rate-limiting step in cholesterol biosynthesis, resulting in lowered blood cholesterol [1,2]. In addition, lovastatin has been used in the biomedical applications such

as, in treating coronary heart diseases, renal diseases, Alzheimer's disease, bone fractions [3]. Moreover, it has been indicated as a potential therapeutic agent for the treatment of various types of tumors because of its capability to suppress tumor growth *in vivo* through inhibition of the synthesis of non-sterol isoprenoid compounds [4, 5].

Commercial production of lovastatin has been carried out by submerged fermentation using different microorganisms [6, 7]. In recent

years, researchers have shown an increasing interest in solid state fermentation (SSF) as it is a potential alternative of submerged fermentation because economical and cheap substrates can be utilized in SSF for production of value-added products, and also requires less energy, and low capital costs. Now days, SSF has been exploited for the production of highly potent drugs on large scale. But still, few reports are available on the microbial production of lovastatin under SSF [8-10].

In the present study, we evaluated the feasibility of SSF process for lovastatin production by *Aspergillus fischeri* and optimized different process parameters that maximize the lovastatin yield.

Materials and Methods

Microorganism and Inoculum preparation:

The fungal strain *Aspergillus fischeri* NCIM 509 used in this study was procured from the culture collection centre of National Collection of Industrial Microorganisms (NCIM), NCL, Pune. The microbial strain was maintained on Potato Dextrose Agar (PDA) slants containing (g/l) dextrose 20.0 and agar 15.0. Inoculated slants were grown in an incubator at 28°C for 5 days. After that, the

slants were preserved at 4°C and sub-cultured once every four weeks (monthly).

Inoculum preparation:

Inoculum was prepared by adding 10 ml of sterilized 0.1% Tween 80 solution to a well-sporulated *A. fischeri* slant. The spore surface was scrapped with an inoculating loop to suspend the spores in the solution and the obtained spore suspension was used as the inoculum for the fermentation process.

Solid state fermentation (SSF):

Coconut oil cake obtained from the local market of Vijayawada, Andhra Pradesh was used as the substrate in the present study. Five grams of coconut oil cake was dried and grounded to required particle size having both coarse and fine particles in 1:1 ratio (w/w). The substrate was taken in a 250ml Erlenmeyer flask and moistened with distilled water to maintain the moisture content of 50% (v/w) and autoclaved at 121°C (15lb) for 15-20 min, cooled to room temperature and inoculated with 2 ml of the 5 day spore suspension (spore concentration of about 10^7 - 10^8 per ml) of *A. fischeri* under aseptic conditions. The contents of the inoculated flasks were mixed thoroughly and incubated at desired temperature in an incubator for desired length of fermentation time.

Optimization of Process Parameters:

Various process parameters that influence the lovastatin production during SSF were optimized over a wide range. The strategy adopted for standardization of fermentation parameters was to evaluate the effect of an individual parameter and incorporate it at the standard level before standardizing the next parameter. The process parameters optimized in the present study include fermentation time (24-192h), initial moisture content (40-100% v/w), inoculum volume (0.5-4ml), initial pH (4-10), incubation temperature (26-40°C), carbon sources (1% w/v) and nitrogen sources (1% w/v). All the experiments were done in triplicate and the mean values of the lovastatin yield was reported.

Lovastatin extraction:

After the fermentation time, the fermented flasks were dried at 60°C for 2 hrs. To this 20 ml of methanol was added to the flasks to extract lovastatin by keeping them in an orbital shaker at 180 rpm for 2 h. The residue was filtered with filter paper and then centrifuged at 1500 rpm for 15 min. The supernatant obtained was collected and analyzed for quantitative determination of lovastatin [10].

Quantitative analysis of lovastatin:

To 1 ml of the obtained supernatant, 1 ml of 1% Trifluoroacetic acid was added and incubated for 10 min (Lactonization of hydroxyl acid form of Lovastatin). From the solution, 0.5 ml was taken and diluted to 10-100 ml in methanol and its absorbance was read at 238 nm by using UV-Visible Spectrophotometer [11].

$$\text{Concentration of Lovastatin (mg/g)} = \frac{\text{Concentration of Lovastatin (mg/ml)} \times \text{Dilution factor (DF)}}{\text{Amount of substrate taken (g)}}$$

Results and Discussion

Selection of solid substrates in SSF for lovastatin production:

Solid substrates employed in SSF processes are heterogeneous natural raw-materials which are generally insoluble in water and play a

dual role-supply of nutrients to the microbial culture growing and anchorage for the growing cells. In the present study, coconut oil cake was used as the solid substrate for both the fungal sporulation and lovastatin production by *Aspergillus fischeri*. Maximum

lovastatin yield of 6.81 mg/g dry substrate was obtained.

Effect of Fermentation time:

The effect of fermentation time on lovastatin production was studied by incubating the fermentation flasks from 24-192 h. The maximum lovastatin yield of 7.43 mg/g of dry substrate was achieved at 168 h of fermentation time (Fig.1). The lovastatin yield increased from 24-168 h which explained that lovastatin is a kind of secondary metabolite and its accumulation in mycelia seems growth related and slightly decreased from 168-192h. The lovastatin yield decreased after seven days (168 h) of fermentation which may be due to the onset of death phase of microorganism and nutrient depletion.

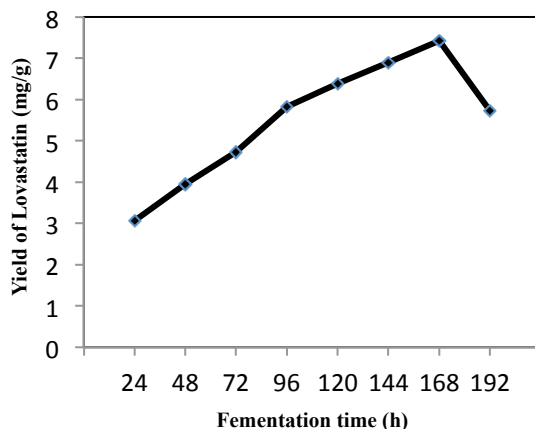


Fig.1: Effect of fermentation time on lovastatin production

Effect of initial moisture content

To investigate the influence of initial moisture content of the substrate, fermentation was carried out under various initial moisture content levels (40, 50, 60, 70, 80, 90 and 100(% v/w)) of coconut oil cake, which was adjusted with moistening media. Maximum lovastatin yield (8.04 mg/g dry substrate) was achieved at 60% (v/w) initial moisture content (Fig. 2.).

As the moisture content in the fermentation medium increases, the air present in the void volume decreases, resulting in poor oxygen availability for the process without forced aeration and with low moisture content, the available oxygen is sufficient but the water content is not enough to support good metabolic activity and dissipation of heat generated and may account for lower lovastatin production. The same 60% (v/w) moisture content was also observed for both *Aspergillus flavipes* and *Aspergillus terreus* under SSF [8, 10].

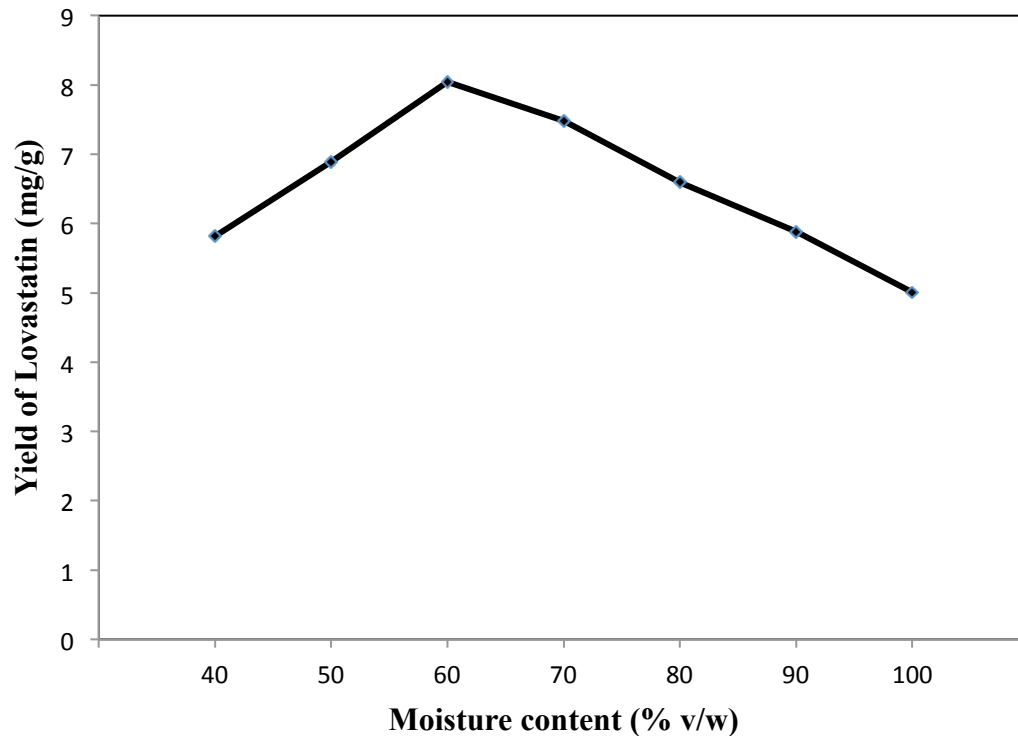


Fig. 2: Effect of moisture content on lovastatin production

Effect of Inoculum volume

Fermentation was carried out with different inoculum volumes (0.5-4ml) of five day old *A. fischeri* culture for a period of 144h to study its effect on lovastatin production. The maximum yield of lovastatin (8.97 mg/g dry substrate) was obtained with 2ml of *A. fischeri* culture as shown in Fig. 3.

With the further increase in inoculum volume, the yield of lovastatin had decreased which might be due to the depletion of the available nutrients in the production medium, yielding poor mycelia growth, thus promoting less product formation. With low inoculum volume, the yield is also low due to the insufficient microbial culture to form mycelia and produce lovastatin.

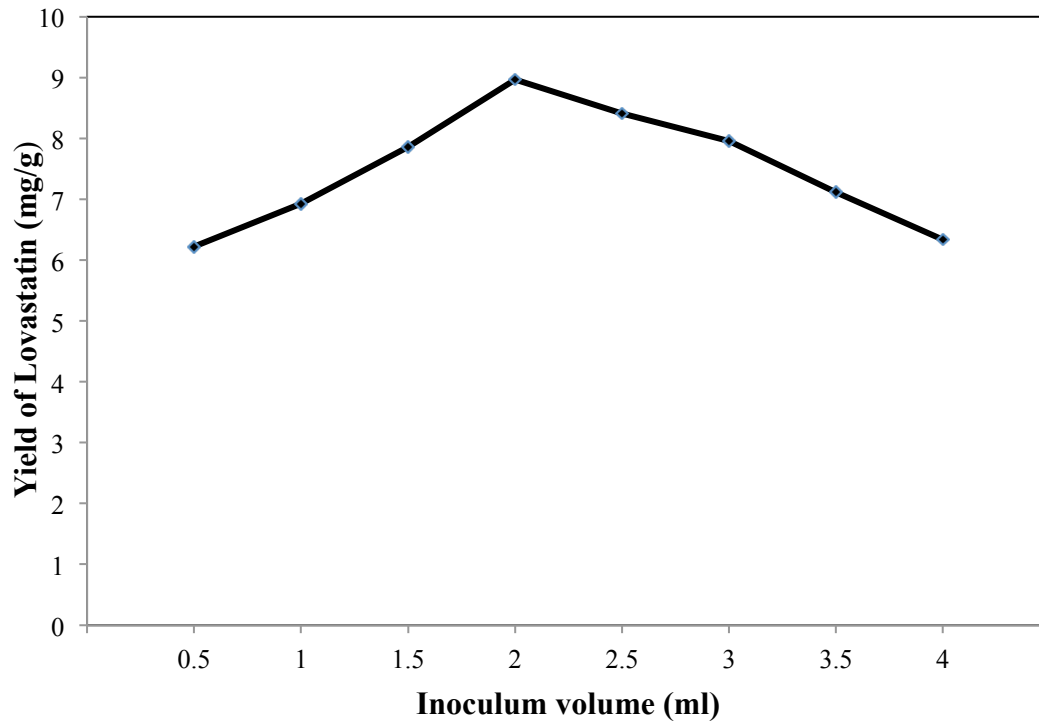


Fig. 3: Effect of inoculum volume on lovastatin production

Effect of initial pH:

Experiments were performed to the optimum pH in order to maintain the favorable conditions for increased production of lovastatin. This was established by carrying out the fermentation by varying the pH from 4-10.

The profound effect of initial pH of the fermentation on lovastatin production was as shown in Fig. 4. Maximum lovastatin yield

(9.71 mg/g dry substrate) was recorded at pH 5.0. A further increase in pH resulted in gradual decrease of lovastatin production due to the denaturation or inactivation of the microbial strain, because pH strongly influences the transport of various components across the cell membrane which support the cell growth and product formation, and most of the fungi are active in the pH range of 3.5-7 and also lower pH avoids the contamination by other microbes. The result was in accordance with the lovastatin production using *Aspergillus terreus* under SSF [8].

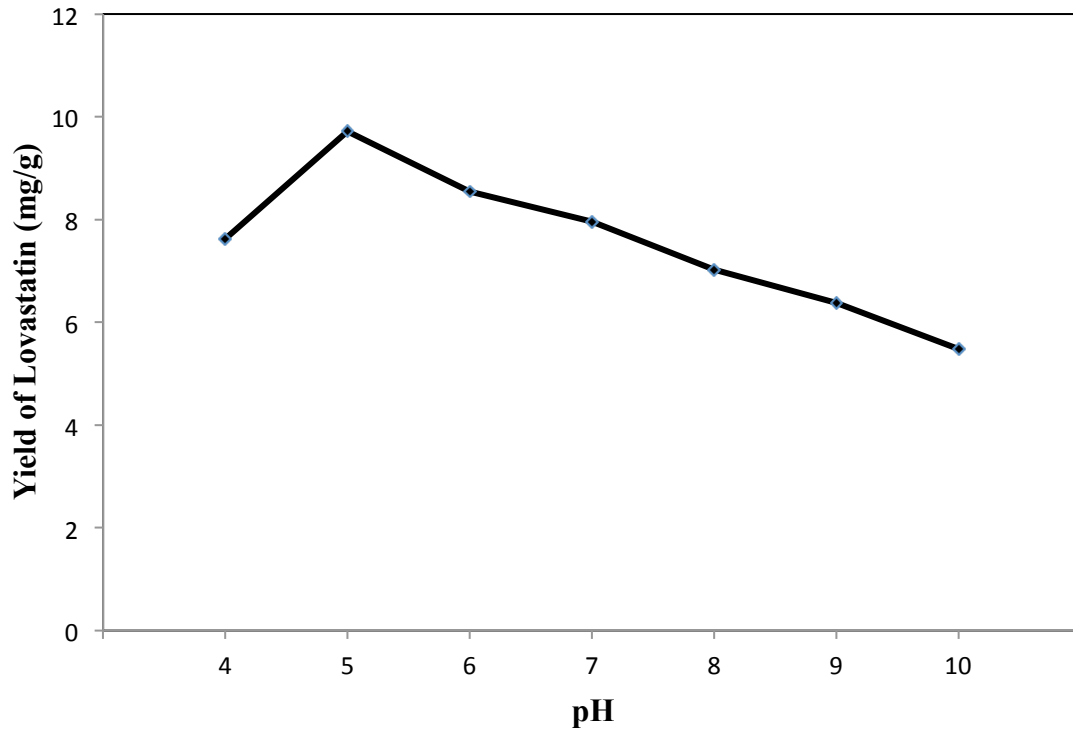


Fig. 4: Effect of pH on lovastatin production.

Effect of temperature

Fermentation was carried out at various temperatures from 26-40°C to study their effect on enzyme production (Fig. 5). Results indicated that maximum lovastatin production (10.88 mg/g dry substrate) was obtained when SSF was carried out at 30°C.

However, lovastatin production reduced gradually above the optimal incubation temperature of 30°C. With further increase

in temperature, more heat is accumulated in the medium during mesophilic aerobic SSF, which leads to poor heat dissipation thus reducing the oxygen level and thereby reducing the growth of microorganism, as lovastatin is growth related product. These results are coinciding with those previously reported for lovastatin production by *Aspergillus terreus* and *Monascus ruber* [10,13].

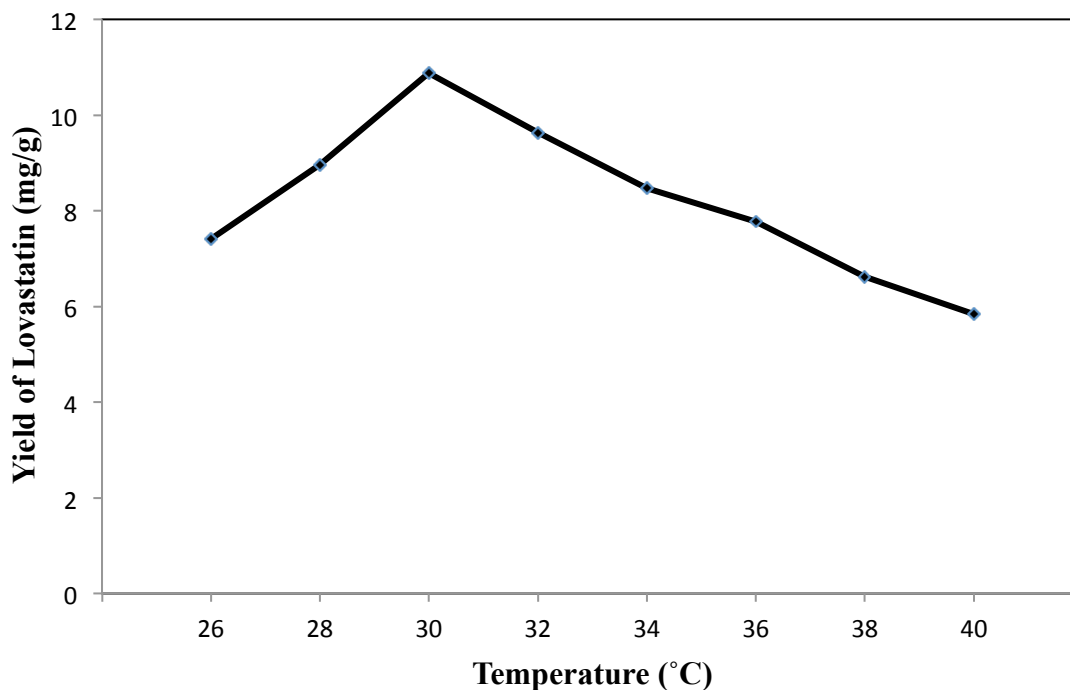


Fig. 5: Effect of temperature on lovastatin production

Effect of carbon source

To determine the effect of incorporation of additional carbon sources on lovastatin yield, different carbon sources namely glucose, maltose, sucrose, xylose, lactose, galactose and soluble starch at 1% (w/v) added to the basal solid state fermentative medium of *A. fischeri* exerted a considerable effect on the biosynthesis of lovastatin (Fig. 6). Maximum lovastatin production was promoted by lactose (12.02 mg/g dry substrate) followed by maltose (11.11 mg/g dry substrate) and sucrose (10.47 mg/g dry substrate). The enhanced production of lovastatin by the incorporation of lactose to

the medium might be attributed to the positive influence of lactose as a co-metabolic agent for enhanced microbial metabolite biosynthesis.

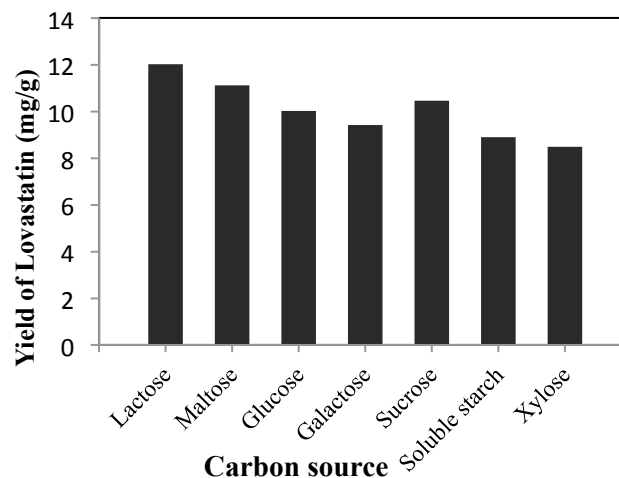


Fig. 6: Effect of different carbon sources on lovastatin production.

Effect of Nitrogen source

Nitrogen source can be an important limiting factor in the production of microbial metabolites [14]. The supplementation of additional nitrogen sources (either organic or inorganic) such as sodium nitrate, ammonium sulphate, urea, peptone, tryptone, yeast extract, malt extract and beef extract at 1% (w/v) had shown a profound impact on the production of lovastatin by *A. fischeri* under SSF (Fig. 7).

Among the various nitrogen sources evaluated, malt extract in the fermentation medium promoted enhanced fungal growth and consequent lovastatin production of 14.77 mg/g dry substrate was achieved followed by yeast extract (12.36 mg/g dry substrate) and sodium nitrate (11.03 mg/g dry substrate).

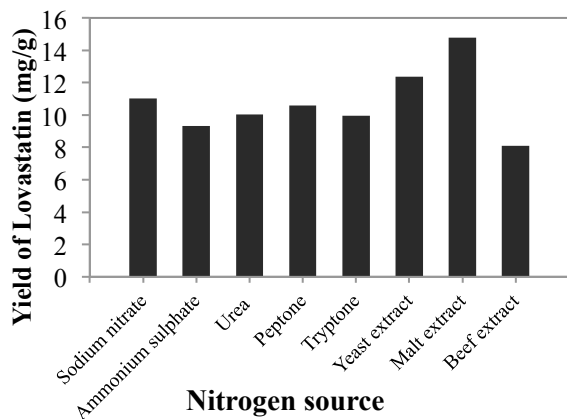


Fig. 7: Effect of different nitrogen sources on lovastatin production

Conclusion:

The current investigation was mainly focused on the evaluation of the potentiality of *Aspergillus fischeri* for utilization of coconut oil cake as the substrate for the production of lovastatin under solid state fermentation. The yields obtained in the present study have to be further increased for its industrial importance. It has proved the feasibility of solid state fermentation as a promising technique in exploiting cheaply available agro-residual wastes as substrates for the large-scale production of microbial metabolites of biotechnological importance ultimately leading to an effective solid waste management.

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