Original Research Article
Biosynthesis of Lovastatin, an anti-cholesterol drug by *Aspergillus wentii* NCIM 661 from Palm kernel cake via Solid-state fermentation

ABSTRACT

This investigation presents the biosynthesis of an anti-cholesterol drug, lovastatin from palm kernel cake (PKC), a by-product obtained during the palm oil processing as a potential substrate, using *Aspergillus wentii* NCIM 661 under solid state fermentation (SSF). All the crucial process parameters such as initial moisture content, pH, incubation temperature, fermentation time and the effect of additional nutritional sources were optimized using single-parameter optimization to enhance the lovastatin production. A yield of 2.71 mg of lovastatin per gram dry substrate was obtained with palm kernel cake under the optimized fermentation parameters respectively. This study successfully and productively utilized the agro-waste and fungal strain for the biosynthesis of lovastatin at their best and demonstrated the feasibility of solid-state fermentation for the commercial production of metabolites of therapeutic significance. Findings from this study are very much promising for the economic utilization and value addition of these important agro residues, which are abundantly available in many developing countries like India.

Keywords: Aspergillus wentii; Lovastatin; Palm kernel cake; Solid-state fermentation.

1. INTRODUCTION

Lovastatin ($C_{24}H_{36}O_5$; also known Monacolin K or Mevinolin) belongs to the class of natural statins, which is most widely and effectively used to control hypercholesterolemia (accumulation of cholesterol in blood plasma). It competitively inhibits 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis [1-3]. Of all the statins available, lovastatin was the first natural statin approved by United States Food and Drug Administration (USFDA) in the year 1987 [4-5]. Moreover, lovastatin has also been reported to possess other therapeutic applications such as an anti-tumor agent against various forms of cancer, anti-inflammatory activity and also plays a vital role in the prevention of neurological disorders; bone disorders etc [6-8].

Lovastatin is mostly biosynthesized from various fungal genus and species as a secondary metabolite. Several fungal genera such as *Aspergillus*, *Monascus*, *Phoma*, *Penicillium Trichoderma*, *Pleurotus*, *Hypomyces* are reported as potential lovastatin producers [9, 10]. Earlier, commercial production of lovastatin by fungi was achieved by employing submerged fermentation (SmF) using *Aspergillus terreus* [11]. Over the past few years, solid-state fermentation (SSF) has emerged as an alternative to submerged fermentation, because of several advantages it offers such as easy control of process contamination, requires fewer processing and down-streaming stages, utilizes lesser power and generates lesser effluent [12]. Another important feature of SSF is its ability to use inexpensive substrates in the form of agro-waste residues for the production of valuable metabolites of industrial importance [13]. Very limited documented reports are available on lovastatin production under solid-state fermentation [14-22].

Palm kernel cake (PKC) is the by-product of oil palm industry; generated after the processing of oil from kernel. It is nutritionally rich containing (% w/w) dry matter 90; crude protein 16.1; ether extract 0.8; crude fibre 15.2; Ash 4; N-free extract 63; calcium 0.29; phosphorous 0.71 and metabolized energy 6.2 [23]. In the current work, the potentiality of palm kernel cake (PKC) was evaluated to see whether the residual waste could be used as a promising substrate in SSF using the fungal strain Aspergillus wentii NCIM 661 for the biosynthesis of therapeutic drug-lovastatin. To the best of our knowledge, this is the first paper reporting palm kernel cake for lovastatin production using Aspergillus wentii NCIM 661.

2. MATERIAL AND METHODS

2.1 Chemicals

Used chemicals are of analytical grade and were purchased from Loba Chemie Pvt. Ltd, Mumbai, India. Culture media was purchased from Hi-media Laboratories, Mumbai, India.

2.2 Microorganism and Inoculum preparation

Fungal strain, *Aspergillus (A.) wentii* NCIM 661, received from NCIM, Pune was used in the present study. The culture was maintained on potato dextrose agar (PDA) slants at 28°C, stored at 4°C and sub-cultured monthly. To a well-sporulated slant of A. wentii, 10 ml of sterilized Tween-80 solution (0.1%) was added. The spore surface was dislodged with an inoculation needle and agitated thoroughly using cyclomixer to suspend the spores uniformly. This was used as inoculum throughout the study.

2.3 Substrate

Palm kernel cake (PKC) was collected from nearby palm oil processing mill in Guntur, Andhra Pradesh, India. Before use, the substrate was sun-dried to remove any extra moisture content and sieved to particle size of 0.5 mm. The substrate was used in SSF without any pre-treatment.

2.4 Solid-state fermentation

PKC (5g) was taken in to 250 ml Erlenmeyer flasks. The moisture content of the media was maintained at 60% using the moistening medium. The contents in the flasks were autoclaved at 121°C (15 lb) for 20 min, cooled to room temperature and inoculated with 1 ml of the fungal spore suspension. The contents were uniformly mixed thoroughly and incubated at 28°C in an incubator for desired period of time (i.e. one week).

2.5 Lovastatin extraction and assay

After completion of fermentation time, the flasks were dried at 40°C for 24 h and crushed into powder form. About 2g of the powdered material was taken and extracted with 100 ml of methanol: water (1:1, v/v) mixture (pH 7.7) in 250 ml Erlenmeyer flask and keeping the flasks at 30°C in rotary shaker at 180-200 rpm for 2 h. After 2 h, the mixture was centrifuged at 10,000 rpm for 10 min and the supernatant was filtered through 0.45 μm membrane filter. The obtained filtrate was collected in vials and preserved at 4°C for further analysis. Lovastatin in the clear extract was estimated by high performance liquid chromatography (HPLC) using a C_{18} column (250 mm x 4.6 mm x 5 mm internal diameter). A mixture of 0.02 M phosphate buffer (pH 7.7) and acetonitrile in the ratio of 65:35 (v/v) was used as mobile phase. The mobile phase flow rate was maintained at 1.0 ml/min and lovastatin was detected at 238 nm with an injection volume of 20 μL [14]. The production of lovastatin is expressed in mg/g dry weight substrate (gds). The yield of lovastatin was calculated [24]. The obtained lovastatin yield was expressed as milligram per gram of the dry substrate (mg/gds).

2.6 Optimization of fermentation conditions

All the essential physicochemical and nutritional variables that influence the lovastatin yield were optimized using single-parameter optimization over a wider range. The parameters such as moisture content (40-80%), pH (4-11, adjusted with 1N HCl/NaOH), incubation temperature (22-40°C), fermentation time (24-168 h). In addition, the impact of various carbon sources (glucose, lactose, maltose, fructose, sucrose, soluble starch, xylose, and cellulose) and nitrogen sources (ammonium sulphate, ammonium nitrate, yeast extract, malt extract, urea and peptone) were also assessed.

3. RESULTS AND DISCUSSION

Since one of the primary motivations for SSF bioprocesses is its economical advantage in utilizing cost-effective agro-wastes for the production of valuable metabolites. So, based on its availability and nutritional factors, palm kernel cake was chosen as a substrate in this SSF to carry out optimization experiments for lovastatin yield enhancement.

3.1 Optimization of fermentation time

The maximum lovastatin yield of 1.19 mg/g of dry substrate was achieved after 72 h of fermentation time (Fig.1). The lovastatin yield increased up to 72 h which explained that lovastatin is a kind of fungal secondary metabolite and its accumulation in mycelia seems growth relatedness.

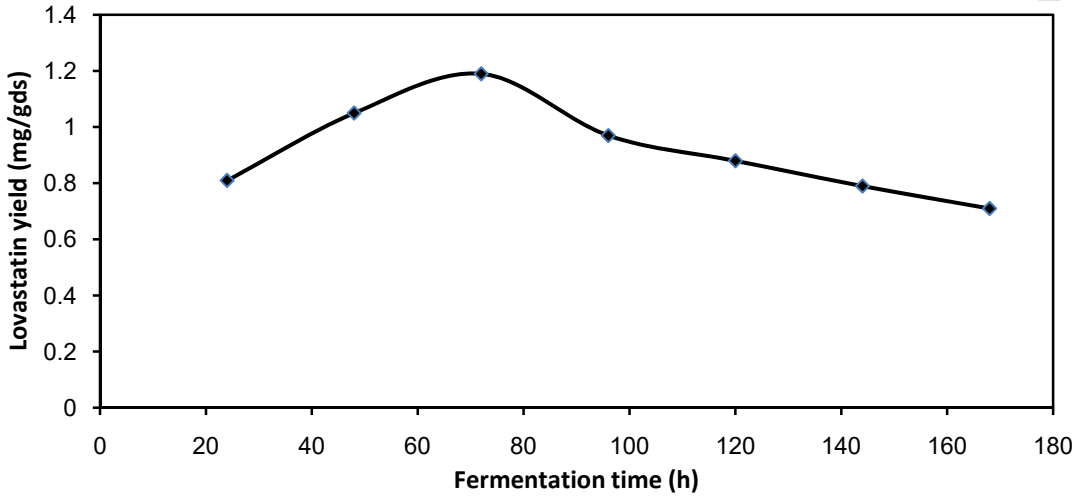

Fig. 1. Effect of fermentation time on lovastatin yield. The maximum yield obtained after 72 h of fermentation time.

Thereafter, a drastic decrease in lovastatin yield was noticed after 96 h. This might be due to the onset of death phase of fungal strain and also depletion of available nutritional sources.

3.2 Optimization of moisture content

Optimal yield of lovastatin (1.62 mg/g dry substrate) was achieved at 60% moisture content (Fig. 2.). Moisture content is one of the critical factors that determine the success of SSF.

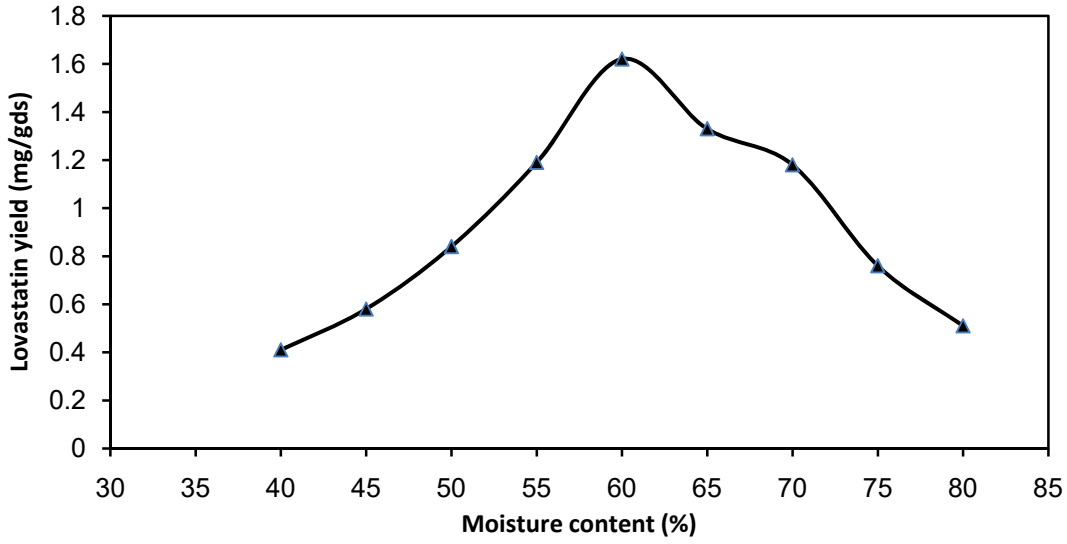

Fig. 2. Effect of moisture content on lovastatin yield. At 60%, the lovastatin yield was maximum

As the water content in SSF medium increases, the air present in the void volume decreases, resulting in poor oxygen availability 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% moisture content was also observed with A*spergillus fischeri* under SSF [15].

3.3 Optimization of initial pH

The profound effect of initial pH on the lovastatin production was as shown in Fig. 3. Maximum lovastatin yield (2.02 mg/g dry substrate) was recorded at pH 7.0.

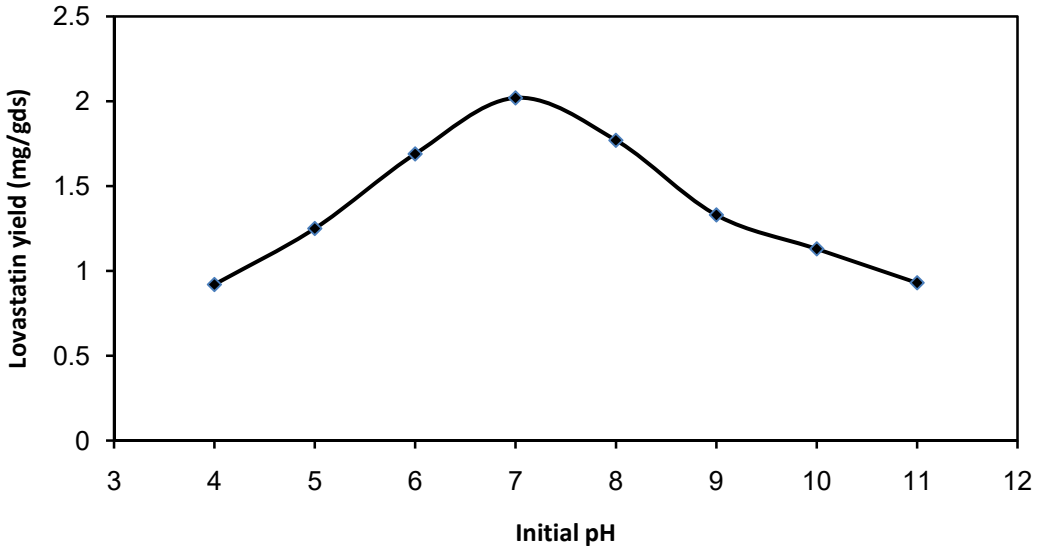

Fig. 3. Effect of initial pH on lovastatin production. At pH 7.0, the yield of lovastatin was optimal

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.

3.4 Optimization of incubation temperature

Results indicated that maximum lovastatin production (2.47 mg/g dry substrate) was obtained when SSF was carried out at 30°C.

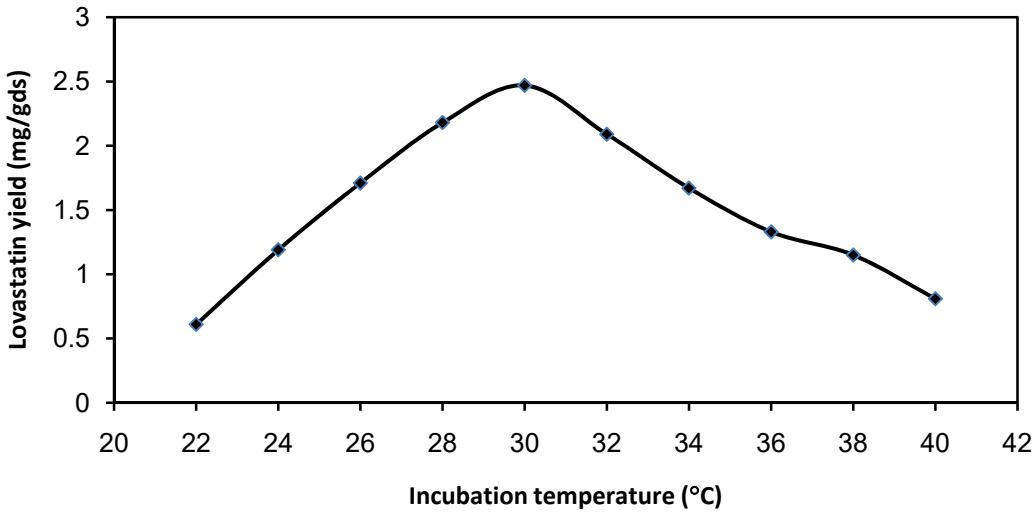

Fig. 4. Effect of incubation temperature on lovastatin production. The lovastatin yield was high at 30°C

However, lovastatin yield gradually reduced after optimal incubation temperature of 30 °C. With further increase in temperature, more heat is accumulated in the medium during aerobic SSF, which leads to poor heat dissipation thus reducing the oxygen level and thereby reducing the growth of microorganism, as lovastatin is a growth related product of fungi. These results are coinciding with those previously reported for lovastatin production by *Monascus ruber* [19].

3.6 Effect of nutritional sources

The nutritional (both carbon and nitrogen) sources were supplemented to the SSF medium in the range of 0.25-2.0% (w/w). Other than, glucose as carbon sources at 0.5%, none of the nutritional sources had shown profound impact on the microbial growth and lovastatin yield (data not presented). The optimal yield of lovastatin reported was 2.71 mg/g dry substrate. The reason might be due to the fact that the utilized substrate, palm kernel cake is already a source of energy and protein which is self-sufficient in nourishing the fungal strain without any external nutrient requirement.

4. CONCLUSION

The finding from this study, clearly demonstrated the lovastatin production process based on palm kernel cake as a potential substrate in SSF is economically feasible and attractive as it is a cheap and readily available agro-residual byproduct in India. This result is of significant interest due to the productive utilization of low cost and abundant availability of residues.

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