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Biosynthesis of Lovastatin, an Anti-cholesterol Drug by *Aspergillus wentii* NCIM 661 from Palm Kernel Cake via Solid-state Fermentation

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CP and SM designed the study, carried out literature searches, performed the statistical analysis and wrote the first draft of the manuscript. Author GP carried out the laboratory work and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Over the past few years, the utilization of various agricultural residual wastes for the production of bioactive metabolites of industrial significance has been increased under solid-state fermentation in converting waste to wealth. In this context, present 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 both 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 with therapeutic

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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; mevinolin; lovastatin; optimization; 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* [11-14]. 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 [15,16]. 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 [17-19]. Very limited documented data is available on lovastatin production under solid-state fermentation using various microbial genera [20-32].

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 [33]. 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 *A. 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 *A. wentii* NCIM 661.

2. MATERIALS 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 National Collection of Industrial Microorganisms (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% v/v) was added. The spore surface was dislodged with an inoculation needle and agitated thoroughly using cyclomixer (REMI: CM-101 PLUS, Mumbai, India) 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 (5 g) 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 2 g 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 [20]. The yield of lovastatin was calculated [34]. 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. All the SSF experiments along with analytical assays were run in three sets and the results reported were mean value of the three sets and standard deviation was ±<5%.

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 the chemical and nutritional factors, cost and availability, palm kernel cake (PKC) was chosen as a promising substrate in this SSF to carry out optimization experiments for lovastatin 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.

After 96 h, there is a drastic decrease in lovastatin yield by the fungal strain. The reason for this decrement might be due to the onset of microbial death phase or the micro organism has attained a stage, from which it could not balance its steady growth with the available nutritional sources [35].

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. Other than the optimized value, metabolite yield was lower on either side. With an increase in moisture level, there is a decrease in porosity, change in the substrate particle structure, lowers oxygen transfer, enhancement of bacterial growth and formation of aerial mycelia [36]. On the other hand, lower moisture content reduces the solubility of substrate nutrients, lowers degree of swelling and higher water tension [37]. The same 60% moisture content was also observed with A*spergillus fischeri* under solid-state fermentation using coconut oil cake [25].

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.

pH beyond 7.0 resulted in the reduction of lovastatin production gradually due to the inactivation of the fungal strain, because pH

strongly influences the transport of various components across the cell membrane and in turn support the cell growth and product formation. Basically agro-residual wastes utilized in SSF possess excellent buffering capacity. Most of the fungal species are active in the pH range of 3.5-7 and also lower pH avoids the contamination by other microbes [35,38].

3.4 Optimization of Incubation Temperature

Results indicated that maximum lovastatin production (2.47 mg/g dry substrate) was obtained when SSF was run at 30°C (Fig. 4). However, lovastatin yield reduced after optimal incubation temperature of 30°C. Generally, most of the fungal strains grow well in a temperature range between 25-32°C and any variation in their growth temperature results in poor metabolite production [39]. Moreover, higher temperatures could lead to poor growth of microorganisms during fermentation due to the thermal denaturation of microbial bio-actives for metabolic pathways [40]. These results are coinciding with those previously reported for lovastatin production by *Monascus ruber* [23].

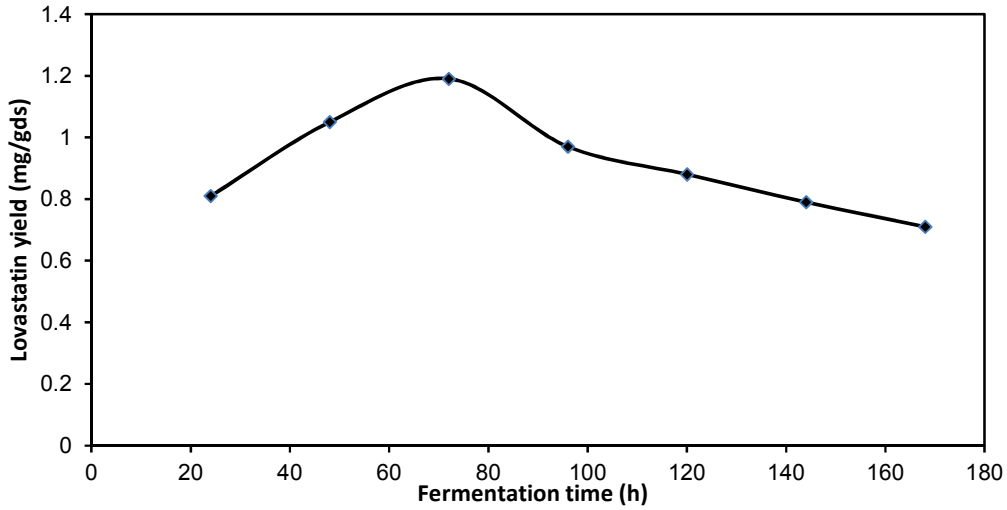

Fig. 1. Effect of fermentation time on lovastatin yield. The maximum yield obtained after 72 h of fermentation time

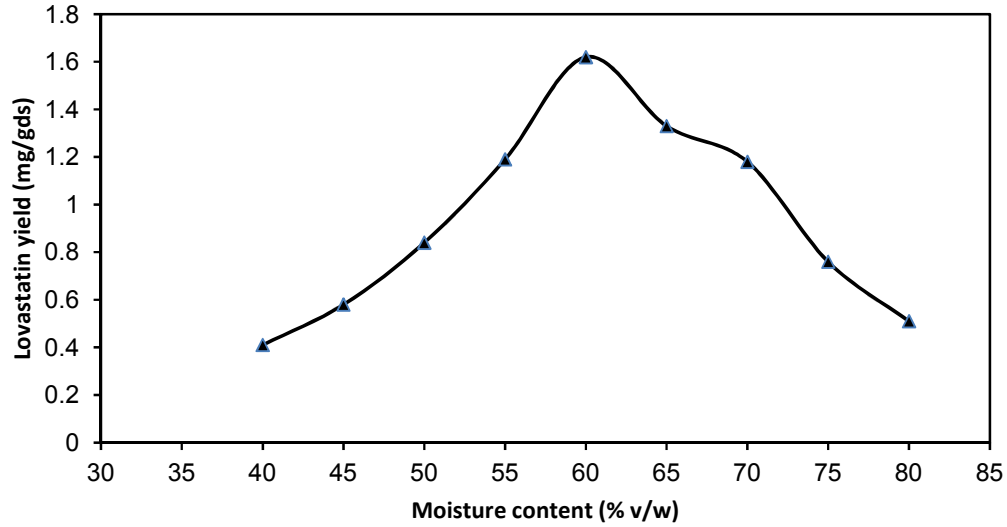

Fig. 2. Effect of moisture content on lovastatin yield. At 60%, the lovastatin yield was maximum

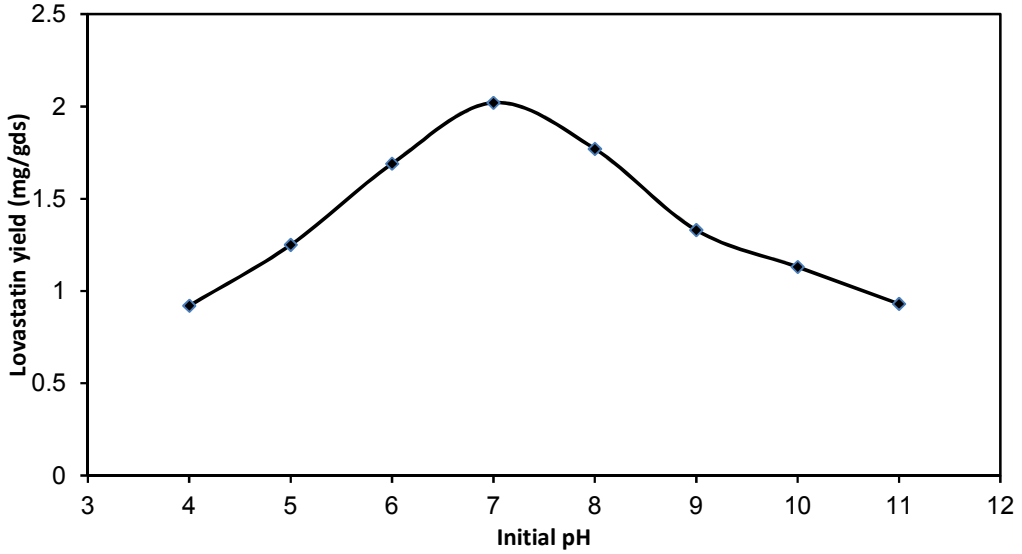

Fig. 3. Effect of initial pH on lovastatin production. At pH 7.0, the yield of lovastatin was optimal

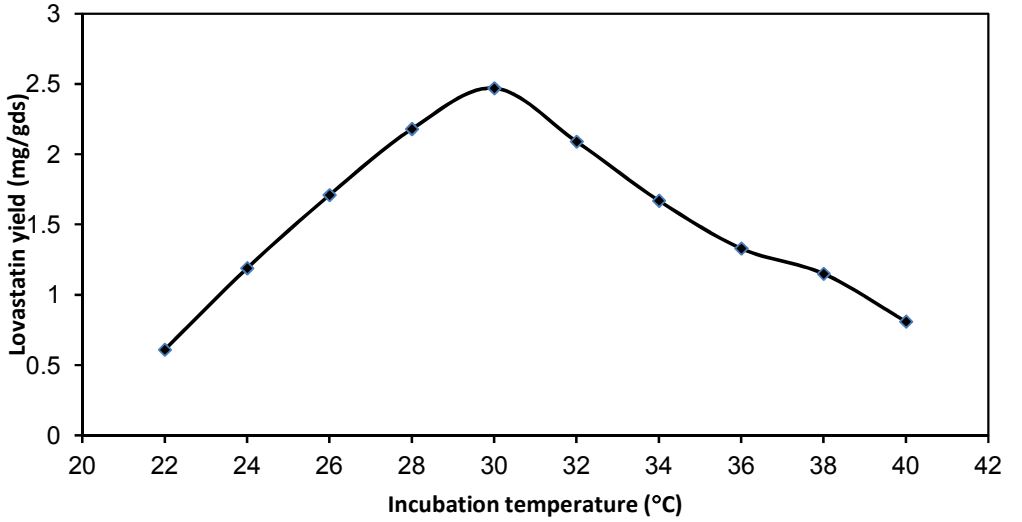

Fig. 4. Effect of incubation temperature on lovastatin production. The lovastatin yield was high at 30°C

3.5 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). None of the nutritional sources, other than glucose as carbon source at 0.5% (w/w), 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 reason that the utilized substrate, palm kernel cake is already a rich source of energy and protein which is self-sufficient in nourishing the fungal strain without any external nutrient requirement.

4. CONCLUSION

The findings from this study clearly demonstrated that 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 for the

production of value-added compounds of industrial importance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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