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Production of Mevastatin by Solid State Fermentation Using Sesame Oil Cake

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ABSTRACT

Statins are the inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, are the class of drugs which are used in the treatment of hypercholesterolemia. They are the most efficient drugs for reducing plasma cholesterol. Mevastatin, also known as compactin is also a statin, catalyzes the conversion of HMG-CoA to mevanolate. Mevanolate is a required building block for cholesterol biosynthesis and mevastatin interferes with its production by acting as a reversible competitive inhibitor for HMG-CoA reductase and thus blocking the enzyme. The present study was carried out on the production of mevastatin by solid state fermentation with *Pencillium citrinum* MTCC 1256 using sesame oil cake. Different agro residues as substrates and different organisms were screened and various physico-chemical parameters like fermentation time, temperature, pH, moisture content were optimized one parameter-at-a-time basis for the production of mevastatin.

Keywords: Mevastatin, solid-state fermentation, *Pencillium citrinum* MTTCC 1256, sesame oilcake, fermentation time, temperature, pH, moisture content.

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INTRODUCTION

One of the major causes of death in developed countries is coronary heart disease. Approximately 10.8% of all deaths are caused due to this disease. Coronary heart disease actually is a wide assortment of diseases. The basic manifestation of many of them is atherosclerosis, caused when fatty deposit called plaque buildup on the inner walls of arteries. Cholesterol is a major component of the atherosclerotic plaque. Many scientists believe that a high level of cholesterol in the blood is a major contributor to the development of atherosclerosis. In humans, the greater part of the cholesterol in the body is synthesized, mostly in the liver, the search for drugs to inhibit cholesterol biosynthesis has long been pursued as a means to lower the level of plasma cholesterol and so it helps to prevent and treat atherosclerosis.

Cholesterol is synthesized from acetyl-coA via a series of more than 20 enzymatic reactions. This synthetic pathway is mainly regulated by the activity of the enzyme 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase, which catalyzes the reduction of HMG-CoA to mevalonate. In many tissues, changes in the activity of this enzyme are closely related to changes in the overall rate of cholesterol synthesis over a wide range of physiological conditions. This enzyme is, therefore, a prime target for pharmacological intervention. Mevastatin, also known as compactin {-[1, 2, 6, 7, 8, 8a-hexahydro-2-methyl-8-(2-methyl butyryloxy) naphthyl]-3-hyroxyheptan-5-olide}, is an optically active compound. Mevastatin is a specific and potent inhibitor of cholesterol biosynthesis, and also acts as an anti-fungal agent [1, 2].Mevastatin is also a precursor of pravastatin, which is also an anti-hpercholesterolemic agent. *Pencillium citrinum* is one of the few commercially used microbial strains for the production of compactin [1]. The molecular formula of mevastatin is $C_{23}H_{34}O_5$ and the molecular mass is 390.The IR spectrum shows hydroxy and lactone absorption, consistent with the formation of a benzoate .The structure of compactin can be divided into two key fragments A hexahydro-napthalene unit as the bottom portion and the lactone unit as the upper portion. The molecule exists in two forms, lactone or acid, and the acidic form is responsible for its biological activity. Mevastatin can be quantitated using HPLC as well as U.V.Spectrophotometer.

MATERIALS AND METHODS

Microorganisms

Penicillium citrinum MTCC 1256 and *Penicillium brevicompactum* MTCC 1999 both procured from MTCC, Institute of Microbial Technology, Chandigarh, India and the slants were maintained on potato dextrose agar (PDA) at 4^{o}C and subculture was done for every three weeks in the laboratory. Fresh slants were prepared for running experiments.

Substrate

Locally available wheat bran, green gram husk, sesame oil cake, coconut oil cake were grounded well and sieved to remove unwanted materials. Initially, all the substrates were screened to determine the potentiality of the above substrates for mevastatin production using SSF method.

Inoculum preparation

Cultures of *Penicillium citrinum* and *Penicillium brevicompactum* was grown on potato dextrose agar (PDA) slants at 30^{o}C and 25^{o}C for 5 and 7 days respectively and maintained at 4^{o}C. Distilled water was added to each slant and the spores were scrapped by using inoculation loop.

Supplement solution

Glucose 5% w/v, Glycerol 12.7 % v/v, Maltose 4 % w/v, KH_2PO_4 1.5 % w/v, $MgSO_4$ 0.5% w/v, Urea 0.6 %w/v, pH 6

Fermentation procedure

Five grams of substrate in total was taken and supplement solution was added to it with initial moisture content being 60% (v/w). The pH of the supplement solution was maintained at 6 using 2N H_3PO_4. All media components were sterilized at 121^{o}C for 15 min and inoculated with 3ml of seed culture. The fermentation was carried out at 26^{o}C for 7 days.

Extraction

At the end of SSF, fermented solid culture was adjusted to pH 6.5 with either diluted acid (aq.H_3PO_4) or alkali (aq.NaOH) and then 25 ml absolute ethyl alcohol was added to it for extraction by keeping in an orbital shaking incubator at 180 rpm for 1h.The residue was filtered with filter paper and then centrifuged at 6000 rpm for 15 min. Then the supernatant was collected and analyzed for quantitative determination of mevastatin.

Quantitative analysis of mevastatin:

1 ml of supernatant was taken and diluted to 10 times with ethanol and 1 ml was taken and diluted again to 10 times with ethanol and incubated for 10 minutes and after that its absorbance was read at 238 nm by using UV-Visible spectrophotometer.

Standard graph:

5mg 99.9% pure mevastatin is dissolved in 50 ml ethanol to give a standard solution of 0.1mg/ml and then various concentrations were made such as 0.01 mg/ml,0.02 mg/ml,0.03 mg/ml to 0.1 mg/ml by diluting to 10 ml by adding ethanol and the absorbance was taken at 238 nm using UV-Visible spectrophotometer. Figure 1 shows standard graph for mevastatin.

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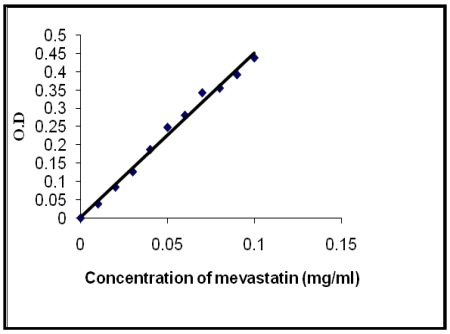

Figure 1: Standard graph for mevastatin

RESULTS AND DISCUSSION

Screening of microorganisms and substrates

Initially, screening for mevastatin production was carried out by employing *Pencillium citrinum* and *Pencillium brevicompactum* strains on four agro-industrial residues. They are sesame oil cake, coconut oil cake, wheat bran, green gram husk and the results were shown in Fig 2. The two fungal cultures produced mevastatin in the range of 0.0108 mg/ml to 0.0196 mg/ml on four substrates. Among them, *Penicillium citrinum* gave better mevastatin production of 0.0196 mg/ml with sesame oil cake closely followed by wheat bran, green gram husk, and coconut oil cake. Finally, sesame oil cake was chosen as the substrate and *Pencillium citrinum* as the organism for production of mevastatin.

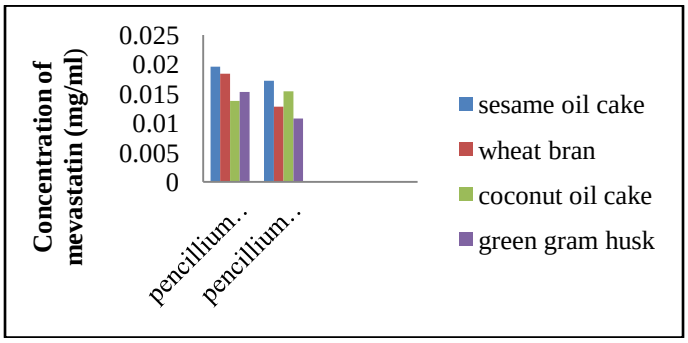

Figure 2: Screening of organisms and substrates for mevastatin production

Optimization of process parameters

Fermentation parameters that influence the mevastatin 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 standard level before standardizing the next parameter. The process parameters optimized in the study include fermentation time, temperature, pH, moisture content.

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Effect of fermentation time

To determine the optimum incubation time for mevastatin production, 5g of substrate autoclaved and moistened with 60% v/w moisturizing medium, pH 6, 3ml inoculum of 6 day old was added and incubated at 26^{o}C temperature varying fermentation time ranging from 1 day to 10 days with an interval of 1 day. The results were shown in Fig 3.The maximum production 0.0266 mg/ml of mevastatin was obtained at 5th day of incubation time, further increase of time decreases mevastatin production and it may be due to onset of death phase of organism and nutrient depletion. M.zafferahamad et al. reported that mevastatin production was maximum at the 6th day of fermentation by using *Pencillium citrinum* from wheat bran under SSF [3].

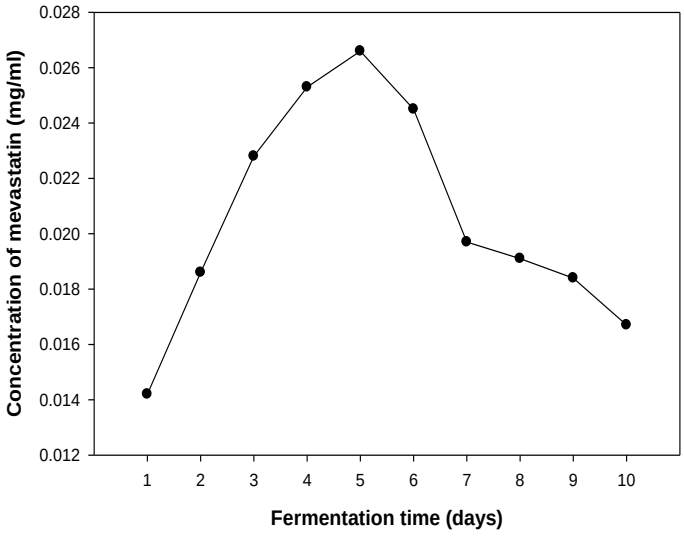

Figure 3: Effect of fermentation time

Effect of temperature on mevastatin production

The effect of temperature on mevastatin production was studied by using, 5g of substrate autoclaved and moistened with 60 %v/w moisturizing medium, pH 6, 3ml inoculum of 6 day old culture was added and incubated at varying fermentation temperatures of 24^{o}C, 26^{o}C, 28^{o}C, 30^{o}C, 32^{o}C, 34^{o}C. The flasks were analyzed after 5 days and the results were shown in Fig 4. Maximum production of 0.0295mg/ml of mevastatin was observed at 28^{o}C temperature and the product was decreased with further increase in temperature. This decrease in the production of mevastatin beyond 28^{o}C may be due to the deactivation of the strain at higher temperatures. Nikhil.S.Shaligram et al., reported highest yield of mevastatin at 27^{o}C by using *Pencillium brevicompactum* from wheat bran and groundnut oil cake under SSF [4].

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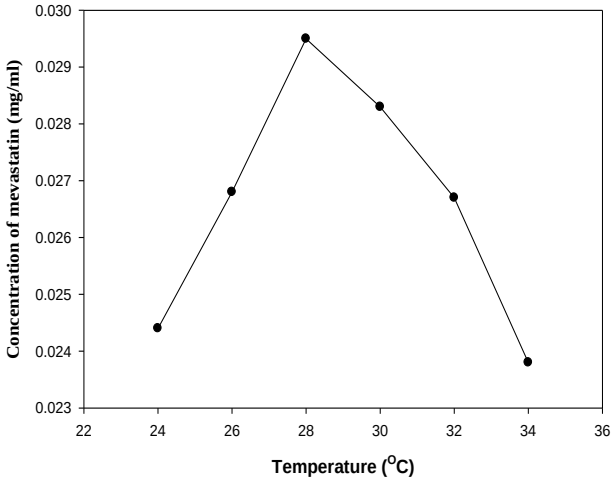

Figure 4: Effect of temperature

Effect of pH on mevastatin production

To determine the effect of pH on mevastatin production, 5g of substrate moistened with 60 %v/w with supplement solution of varying pH at 3.0, 4.0, 5.0, and 6.0, 7.0 and 8.0, and inoculated with 3ml inoculum of 6 day old culture and incubated at 28°C. The flasks were analyzed after 5 days and the results were shown in and Fig 5. Maximum production of mevastatin of 0.0293 mg/ml was obtained at pH 6.0. Most of the fungi grow actively at acidic pH which is necessary for transport of various components across the cell membrane thus strongly influencing the cell growth and secondary metabolites production.

N.S.Shaligram et al., reported highest production of mevastatin at 7.5 pH by using *Pencillium brevicompactum* from wheat bran and groundnut oil cake under SSF [5].

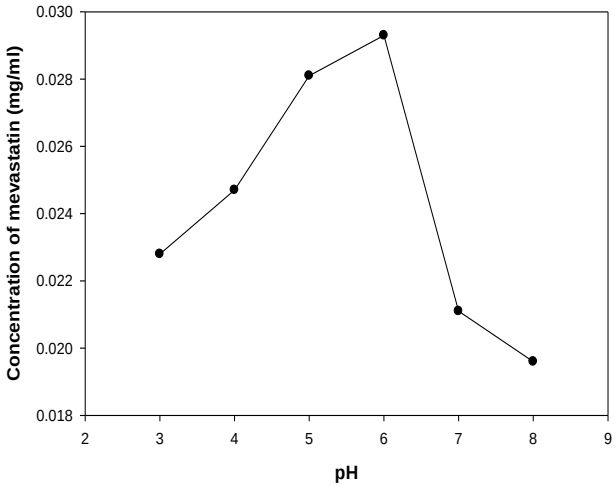

Figure 5: Effect of pH

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Effect of moisture content on mevastatin production

The moisture content has an important role in solid state fermentation, and it has been observed that high moisture content leads to aggregation of substrate particles, poor aeration, and possible anaerobic conditions while very low moisture content restricts the fungal growth. Optimal moisture content depends on the nature of microorganism and the substrate being used [4]

To determine the effect of moisture content on the fermentation medium for mevastatin production, 5g of substrate with varying moisture contents of 20, 40, 60, 80, 100, 120 %v/w with moisturizing medium pH 6 and with 3 ml inoculum of 6 day old culture was incubated at 28^{o}C. The flasks were analyzed after 5 days and the results were shown in Fig 6. Maximum mevastatin production of 0.0294mg/ml of substrate was obtained with moisture content of 60 % v/w. M.zafferahamad et al., reported maximum production of mevastatin at 70% moisture content by using *Pencillium citrinum* wheat bran under SSF [3].

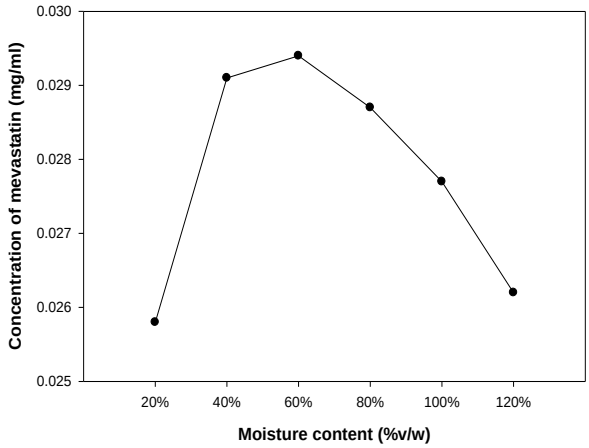

Figure 6: Effect of moisture content

SUMMARY AND CONCLUSIONS

The fungal metabolites, mevastatin and related compounds are potent competitive inhibitors of HMG-CoA reductase enzyme. The present study was carried out on the production of mevastatin by solid state fermentation with *Pencillium citrinum* MTCC 1256 using sesame oil cake. Various parameters like fermentation time (1-10 days), temperature (24-34^{o}C), pH (3-8), moisture content (20-120 %v/w), were optimized one parameter at a time basis. The optimized values of the variables thus obtained were: Fermentation time 5 days, Temperature 28^{o}C, pH 6, Moisture content 60% v/w.

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