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Optimization of Physico Chemical and Nutritional Parameters for the Production of Mevastatin Using *Pencillium citrinum* MTCC 1256

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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 at 28^{o}C. It deals with the study of parameters such as inoculum age, inoculum volume; nutritional suppliments such as carbon source, nitrogen source and their concentrations were optimized for the effective production of mevastatin.

Keywords:- carbon source, inoculum age, inoculum volume, mevastatin, nitrogen source, Pencillium citrinum MTTCC 1256, sesame oil cake, solid-state fermentation.

I. INTRODUCTION

Mevastatin, also known as compactin or ML-236B is a member of the class of statins belonging to the polyketide group. Polyketides are rich sources of pharmaceuticals, including antibiotics, anticancer drugs, and cholesterol-lowering drugs, immunosuppressants and other therapeutics.[1] 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.[2] Mevastatin is a specific and potent inhibitor of cholesterol biosynthesis, and also acts as an antifungal agent.[3] Mevastatin competitively inhibits the regulatory enzyme, 3-hydroxy-3-methylglutaryl-coenzyme- A-reductase. Mevastatin is also a precursor of pravastatin, which is also an anti-hypercholesterolemic agent. Among the few commercially used microbial strains for the production of mevastatin are *P. citrinum, P. cyclopium* and *Aspergillus terreus* [3-5]. In this study we have carried out experiments to optimize the physico chemical parameters and nutritional parameters to improve the production of mevasatin by sesame oil cake.

II. MATERIALS AND METHODS

2.1. 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.

2.2. 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 and sesame oil cake gave the maximum production of mevastatin using SSF method

2.3. 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.

2.4. Supplement solution

The solution was supplemented with 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

2.6. 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 2 N H_3PO_4. All media components were sterilized at 121^{o}C for 15 min and inoculated with 3 ml of seed culture. The fermentation was carried out at 28^{o}C for 5 days.

2.7. 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.

2.8. Quantitative analysis of mevastatin

1 ml of supernatant was taken and diluted to 10 times with ethanol and from this 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.

2.9. Standard graph

5 mg 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 from 0.01 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. Fig 1 shows standard graph for mevastatin.

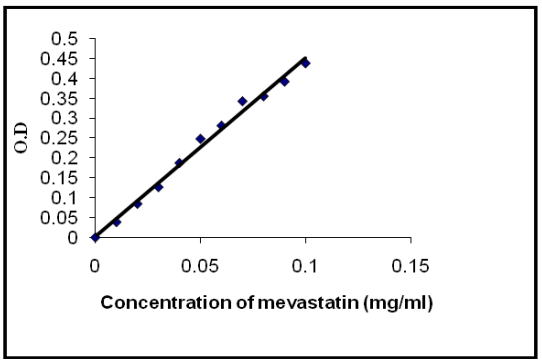

Fig 1. Standard graph for mevastatin

III. RESULTS AND DISCUSSION

3.1. Effect of age of inoculum

To determine the optimum age of inoculum for mevastatin production, 5 g of substrate autoclaved and moistened with 60 % v/w moisturizing medium pH 6.0, 3 ml inoculum was added at 28^{o}C, by varying the age of inoculum from 2nd, 4th, 6th, 8th, 10th, 12th, 14th, to 16th day and the flasks were analyzed after 5 days and the results were shown in the Fig 2.The maximum production of mevastatin of 0.0356 mg/ml was obtained with 12th day old culture. Shaligram et al., (2008)[6] reported highest yield of mevastatin with 12 day old inoculum of *Pencillium brevicompactum* from wheat bran and groundnut oil cake under SSF.

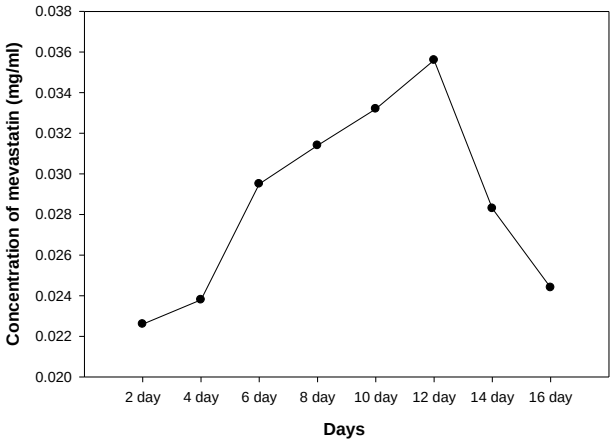

Fig 2: Effect of the age of inoculum for the maximum production of mevasatin

3.2. Effect of inoculum volume on mevastatin production

To determine the effect of inoculum volume on mevastatin production, 5 g of substrate autoclaved and moistened with 60 % v/w moisturizing medium of pH 6.0 with varying inoculum volume of 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, of 12 day old culture was incubated at 28^{o}C. The flasks were analyzed after 5 days and the results were shown in Fig 3. Maximum production of 0.0402 mg/ml of mevastatin was obtained with 5 ml inoculum volume. Maximum production of mevastatin was reported with 2 ml inoculum volume by using *Pencillium citrinum* from wheat bran under SSF by Endo et. al., and zafferahamad et al. [7, 8]

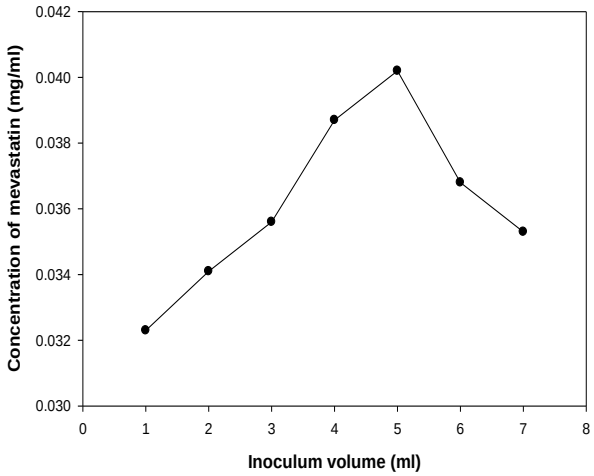

Fig 3: Effect of inoculum volume for the maximum production of mevasatin

3.3. Effect of additional carbon supplementation on mevastatin production

To determine the effect of additional carbon source on mevastatin production, maltose in the supplement solution was replaced with different carbon sources such as maltodextrin, sucrose, maltose, lactose, fructose, starch, xylose at a concentration of 4 %w/v (0.2 g per 5ml of supplement solution) with other optimized conditions applied to the fermentation medium (5g of substrate moistened by 60 %v/w with moisturizing medium of pH 6, 5 ml inoculum of 12 day old culture) and incubated at 28^{o}C for 5 days. Among different carbon sources, maltodextrin gave maximum mevastatin prouction of 0.0472 mg/ml when compared to other carbon sources. The results were shown in the Fig 4.

Glucose and glycerol concentrations were kept constant in the supplement solution. The presence of additional carbon sources such as glucose and maltose supports the initial growth and biomass formation, while glycerol is utilized slowly and helps in later stages of growth. Glucose supplementation was found to be excellent substrate for growth, which could be due to its rapid utilization by the fungal culture. [8] Production of secondary metabolites, has been found to be independent from growth. Shaligram et al., (2008) **[6]** reported highest yield of mevastatin with maltose as an additional carbon source by using *Pencillium brevicompactum* from wheat bran and groundnut oil cake under SSF.

Fig 4: Effect of carbon source for the maximum production of mevastatin

3.3.1. Effect of maltodextrin concentration on mevastatin production

To optimize the concentration of optimized carbon supplement, i.e. maltodextrin, 0.1, 0.2, 0.3, 0.4, 0.5 g of maltodextrin was added to the production medium with other optimized conditions (5g of substrate moistened by 60 % v/w moisturizing medium of pH 6.0 and with 5ml inoculum of 12 day old culture and incubated at 28^{o}C for 5 days). Maximum production of mevastatin of 0.0481 mg/ml was obtained at a concentration of 0.3 g (6%w/v) concentration of maltodextrin. The results were shown in the Fig 5.

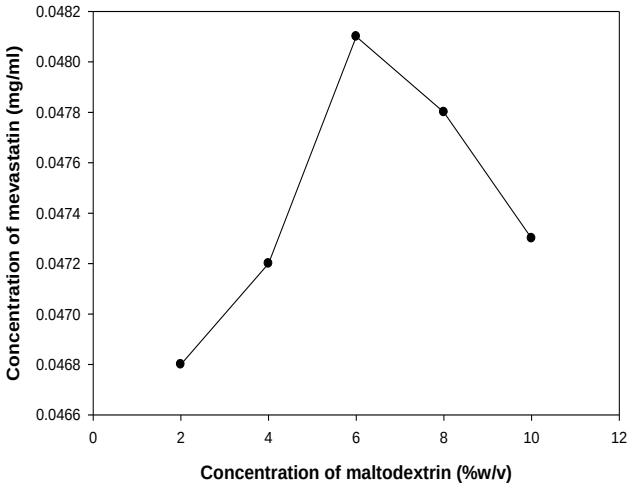

Fig 5: Effect of concentration of maltodextrin for the maximum production of mevastatin

3.4. Effect of nitrogen source mevastatin production

To determine the effect of nitrogen supplementation on mevastatin production, different nitrogen sources such as ammonium sulphate, sodium nitrate, malt extract, urea, ammonium nitrate and yeast extract at a concentration of 0.6 %w/v (0.03 g) with other optimized conditions applied to the fermented medium (5 g of substrate moistened by 60 % v/w moisturizing medium of pH 6.0, 5 ml inoculum of 12 day old culture and incubated at 28^{o}C for 5 days without addition of carbon supplement. Among different nitrogen sources, ammonium nitrate gave maximum mevastatin product of 0.0478 mg/ml when compared to other nitrogen sources. The results were shown in the Fig 6. Shaligram et al.,(2008) [6] reported highest yield of mevastatin with ammonium nitrate by using *pencillium brevicompactum* from wheat bran and groundnut oil cake under SSF.

Fig 6: Effect of nitrogen source for the maximum production of mevastatin

3.4.1. Effect of Ammonium nitrate concentration on mevastatin production

To optimize the concentration of optimized nitrogen supplement, i.e ammonium nitrate 0.01, 0.02, 0.03, 0.04, 0.05g of ammonium nitrate were added to the production medium with other optimized conditions (5g of substrate moistened by 60 % v/w moisturizing medium of pH 6.0 and with 5 ml inoculum of 12 day old culture and incubated at 28^{o}C for 5 days).Maximum production of mevastatin of 0.0517 mg/ml obtained at a concentration of 0.05g (1% w/v) concentration of ammonium nitrate. The results were shown in the Fig 7. [9]

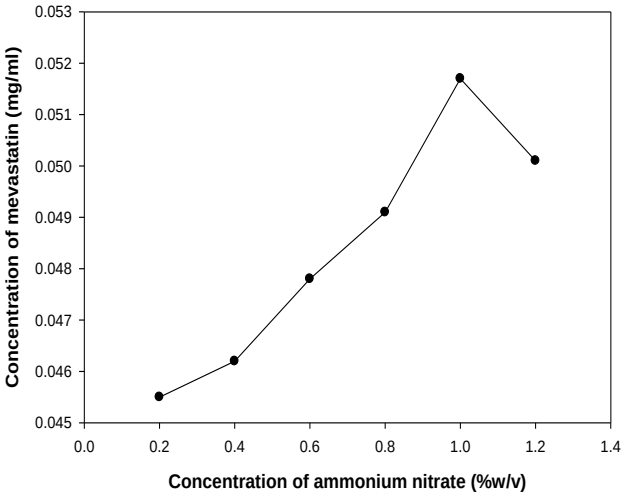

Fig 7: Effect of concentration of ammonium nitrate

IV. SUMMARY AND CONCLUSION

Fungi produce secondary metabolites such as growth factors, anti bacterial and hypocholosteromeric agents. Among them, mevastatin is a highly specific and potent inhibitor of cholesterol biosynthesis, and also acts as an anti-fungal agent. Mevastatin is also a precursor of pravastatin, which is also an anti-hypercholesterolemic agent. In order to reduce the cost of production of mevastain and to know the various factors effecting the production, this work was done under solid state fermentation using agro industrial residues like sesame oil cake using *pencillium citrinum* MTCC 1256. Various parameters inoculum age (2-16 days), inoculum volume (1-7 ml) and concentrations of the nutritional parameters like additional carbon source and nitrogen source were optimized one parameter at a time basis for the maximum production of mevasatin. The optimized values of the variables thus obtained were: Inoculum age - 12 days, Inoculum volume – 5 ml, Maltodextin - 6% w/v, Ammonium nitrate - 1.0 w/v.

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