Optimization of fermentation conditions for lovastatin production in solid state fermentation using Aspergillus terreus KLVB

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ABSTRACT

Lovastatin (also known as mevinolin) is potent competitive inhibitor of the enzyme 3-hydroxy 3-methyl glutry coenzyme A (HMG CoA) reductase, a key enzyme in cholesterol biosynthesis in the body. The present highlights lovastatin production by *Aspergillus terreus* KLVB28 strain using deseeded carob pods as solid substrate. Different parameters like moisture content, pH, temperature, inoculum size, particle size and bed depth were optimized for lovastatin production. The present study revealed that the moisture level of 65%, pH of 4.5, temperature of 35°C, inoculum size of $1x10^8$ spores/ml, particle size of 2 mm and bed depth of 2 cm is suitable for the maximum production of lovastatin under SSF employing *A. terreus* KLVB28, using deseeded carob pods as substrate. The data revealed that maximum yeild of 289.63mg/g of lovastatin was obtained using carob pods as a substrate.

INTRODUCTION

Lovastatin is one of the most potent hypocholesterolemic agent, discovered independently by Endo (1979), and Alberts (1980), from the fermentation broths of *Monascus ruber* and *Aspergillus terreus*, respectively. Generally it is used as a cholesterol lowering agent and it acts by inhibiting the key enzyme, HMG COA reductase responsible for cholesterol biosynthesis (Alberts *et al.*, 1980).

Carob Pod, a fruit of carob tree (*Ceratonia siliqua*) contains nearly 50-60% water soluble sugars and hence it is widely used in the production of Ethanol (Roukas, 1994) and citric acid (Roukas, 1999). The production of lovastatin from carob pods by solid state fermentation has not been reported, but information on production and optimization of condition for lovastatin under solid state fermentation using carob pod by fungi are scanty (Szakacs *et al.*, 1998, Manzoni *et al.*, 1999). Hence, an attempt has been made to study the production of lovastatin by adjustment of various fermentation parameters such as moisture content, pH, temperature, inoculum size, particle size and bed depth.

MATERIALS AND METHODS

Organisms: A strain of *Aspergillus terreus* KLVB28 obtained (local soil) through the *Neurospora crassa*-bioassay method was used under the present study context.

Spore inoculum preparation: Spore suspension of *A. terreus* KLVB28 was prepared from 7 days old culture grown on PDA slants by adding 10 ml sterile water containing 0.01% Tween-80 and suspended the spores with a sterile loop.

Lingappa & Vivek Babu (2004). Pages 291-297.

Solid state fermentation methodology: The flasks containing substrate (20 g) were autoclaved at 121°C for 20 min and cooled to room temperature and then the flasks were inoculated with 2 ml of spore suspension. The contents of the flasks were thoroughly mixed by gently tapping. Thus prepared flasks were incubated at 35°C and kept in slanting position for a period of days.

Optimization of fermentation parameters: The production of lovastatin mainly depends on various factors like moisture content, initial pH, temperature, inoculum size, particle size and bed depth. Hence these parameters were optimized one by one, to achieve higher amounts of lovastatin, in fermentation studies. Once a parameter was optimized, the same optimum condition of that specific parameter was employed in further study where as in another fermentation parameter was to be optimized.

a) Moisture content: A set of conical flasks containing 20g substrate (2mm size) were moistened with an appropriate amount of distilled water in order to obtain different moisture levels like 20%, 25%,3 0%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%. Thus the prepared flasks were inoculated as earlier and fermented.

b) Initial pH: The flasks containing 20g substrate with 65% moisture level were adjusted to different pH levels, that is, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 (Roukas, 1999). Thus prepared flasks were inoculated and incubated as described earlier.

c) Temperature: The fermentation flasks prepared as above were incubated in BOD incubators at different temperatures levels, that is, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C as described by Roukas (1999).

d) Inoculum size: A set of conical flasks containing 20 g of substrate were different levels of spore inoculum, that is, $1x10^5$ to $1x10^{10}$ spores/ml and incubated at 35°C for 7 days in slanting position.

e) Particle size: 20 g substrate of different particle sizes like 2 mm, 4 mm, 6 mm and 8 mm were prepared using sieves of respective pore sizes. Thus prepared substrates, taken in conical flasks were inoculated and incubated in the BOD incubator.

f) Bed depth: The substrate having particle size of 2 mm was taken and adjusted to bed height of 1 cm, 2 cm, 3 cm, 4 cm and 5 cm. Thus the prepared flasks were inoculated and incubated as discussed earlier.

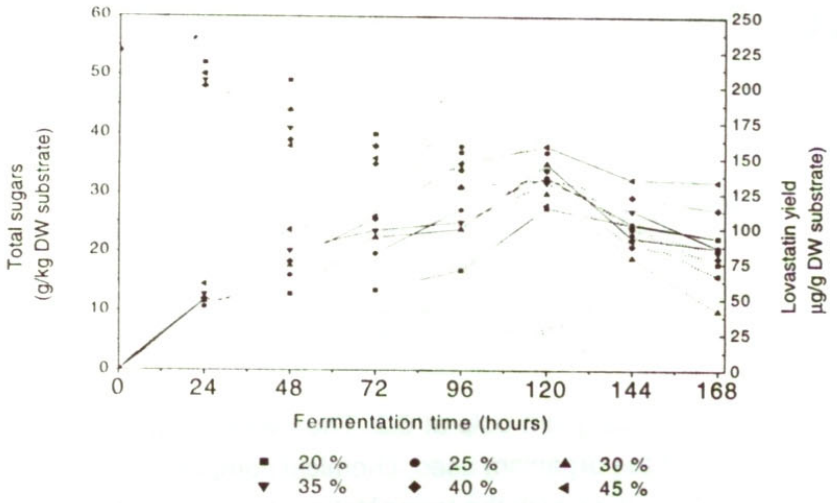
Estimation of Lovastatin and Residual Sugars: The samples were withdrawn periodically for every 24 hr under aseptic conditions and estimated as per Morovjan *et al.* (1997), wherein 1g of moldy substrate was extracted with 10ml acetonitrile and 0.5ml phosphoric acid. The contents were incubated on a orbital shaker for 60 min at 120 rpm and centrifuged. The superntant liquid was analyzed for lovastatin at 238 nm, using UV-Vis spectrophotometer. The amount of lovastatin was calculated by referring standard lovastatin graph. The residual sugars in the fermentation medium were estimated by phenol-sulphuric acid method (Dubios *et al.*, 1956).

RESULTS AND DISCUSSION

In this study, Lovastatin production by *Aspergillus terreus* KLVB 28 strain was undertaken by SSF conditions and cultural parameters were optimized for improving the lovastatin production. The lovastatin production from deseeded carob pods under SSF is shown in fig. 1-6.

a. Effect of moisture content: One important factor that affects the performance of solid state fermentation is the moisture content. The purpose of this experiment was to determine the optimum miosuture level of deseeded carob pod that would result in the highest lovastatin productions. As shown in Fig. 1(a) and Fig. 1(b) lovastatin yield and sugar utilization were increased with the increase in moisture content upto 65%. The

Fig.1(a) Effect of moisture content on the yield of Lovastatin(____) by *A.terreus* KLVB28 and residual sugar concentration(....) in solid state fermentation of carobpods.



highest yield of lovastatin was obtained at a moisture level of 65% and resulted in 218.88 mg/gDW of lovastatin at 120 hrs of fermentation period. These results agree with the results of Szakacs (1998), who reported the lovastatin production in sweet sorghum pulp and wheat straw with optimum of 75% moisture level. Decreasing of moisture levels from 75% to 65% is advantageous in carob pods. Since the chance of contamination in the fermentation medium is reduced however, too low substrate moistures in solid-state fermentations resulted in suboptimal product formation (Roukas, 1999).

b. Effect of initial pH: The effect of initial pH on carob pod fementation reveals that the yield of lovastatin increased with the increase in the initial pH of the substrates upto 4.5 units at 120 hrs of fermentation period and thereafter the oriductin of lovastatin decreases with the increase in the initial pH. The maximum yield of lovastatin (220.18 mg/gDW) was obtained at pH 4.5 and the minimum yield of lovastatin (102 mg/gDW) was observed at pH 2.0, with *A. terreus* KLVB28 strain. Szakacs (1998) reported the maximum production of

Fig.1(b) Effect of moisture content on the yield of Lovastatin(____) by *A.terreus* KLVB28 and residual sugar concentration(....) in solid state fermentation of carobpods.

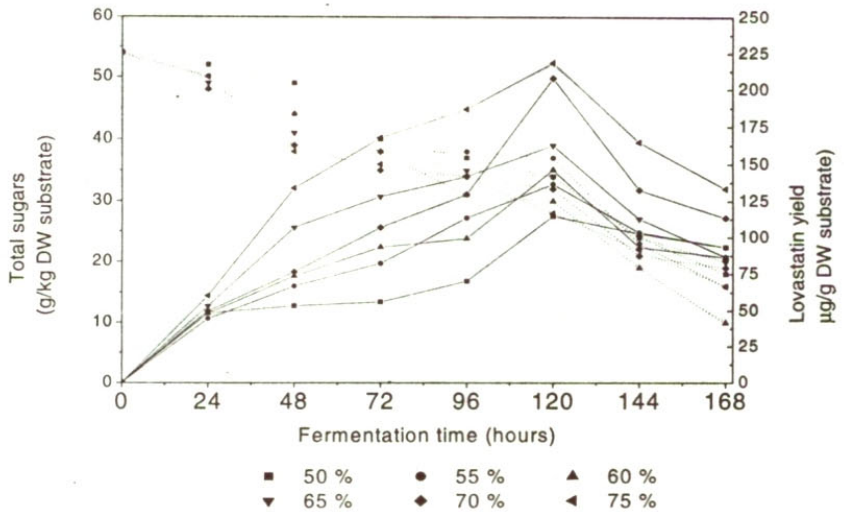
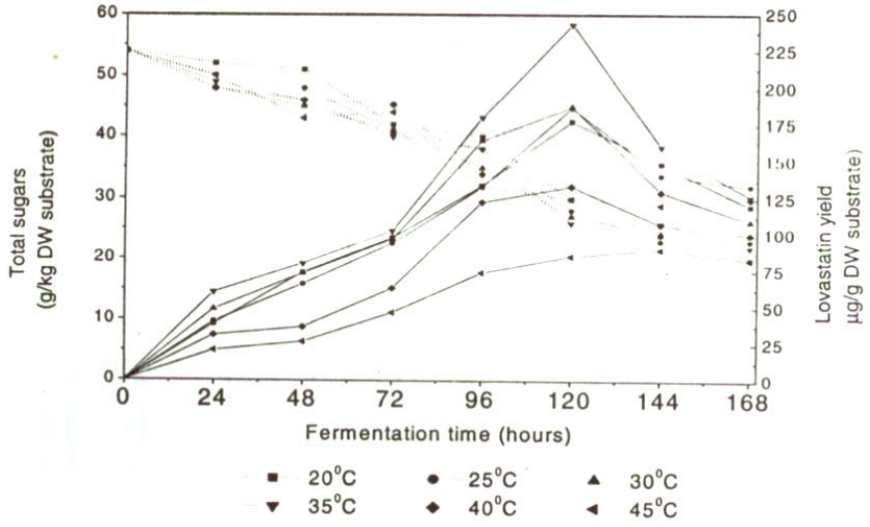


Fig. 3. Effect of temperature on the yield of Lovastatin (_____) by *A. terreus* KLVB28 and residual sugar concentration (....) in solid state fermentation of carobpods



lovastatin in wheat bran and sweet sorghum pulp at 6.2. The slight variation in pH optimum for lovastatin production may be due to strain of the organism used, chemical composition of the substrate, fermentation system and finally, the conditions under which the fermentation takes place (Roukas, 1999).

c. Effect of temperature: The effect of temperature on lovastatin production was shown in Fig. 3. The yield of lovastatin increased significantly with the increase in fermentation temperature from 20°C-35°C and decreased above 35°C. The maximum lovastatin yield obtained at 35°C was 243.33 mg/gDW and the least yield was observed at 45°C resulted in only 76 mg/gDW of Lovastatin at 120 hrs of fermentation period. The decline in the yield of lovastatin at higher temperatures may have been due to decay in the enzyme system responsible for the production of lovastatin upon exhaustion of the fermentable sugars (Hajjaj, 2001). Szakacs *et al.* (1998) reported that the lovastatin production in solid state fermentation using wheat bran and sweet sorghum pulp was maximum at 25°C by employing *Aspergillus terreus* TUB-F 514 strain. Similarly, Manzoni *et al.* (1998)

Fig.4 Effect of inoculum size on the yield of Lovastatin (_____) by *A.terreus* KLVB28 and residual sugar concentration (....) in solid state fermentation of carobpods.

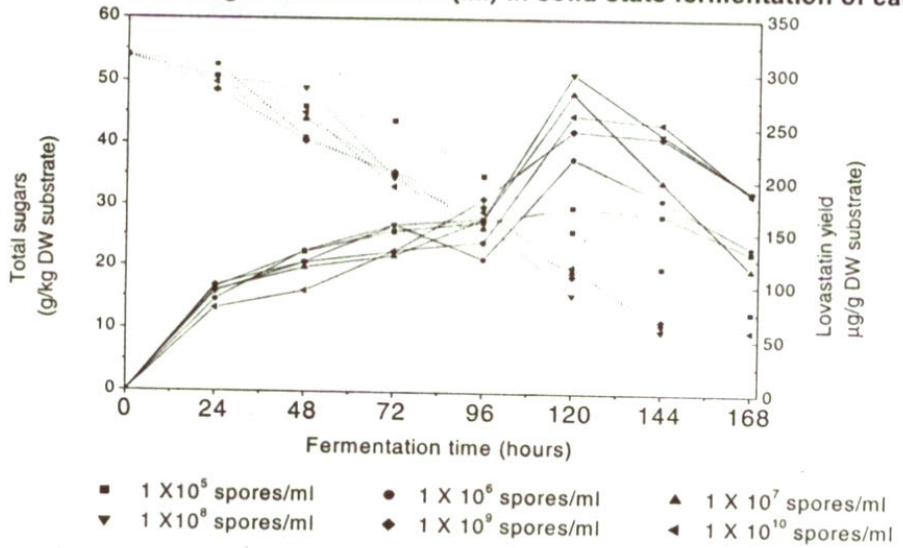
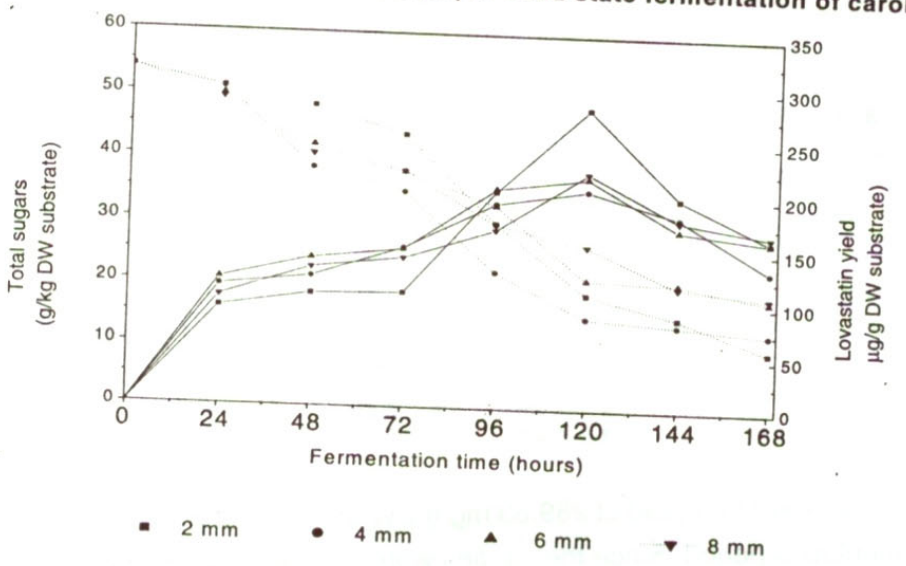


Fig.5 Effect of particle size on the yield of Lovastatin (____) by *A.terreus* KLVB28 and residual sugar concentration (....) in solid state fermentation of carobpods.



studied the production of statins using *Aspergillus terreus* in submerged fermentation systems and reported the optimum temperature for lovastatin production to be 25°C. However, Hajjaj *et al.* (2001) reported 35°C as the optimum temperature for maximum production lovastatin. Hence, our observations were in accordance with the result of Hajjaj *et al.* (2001).

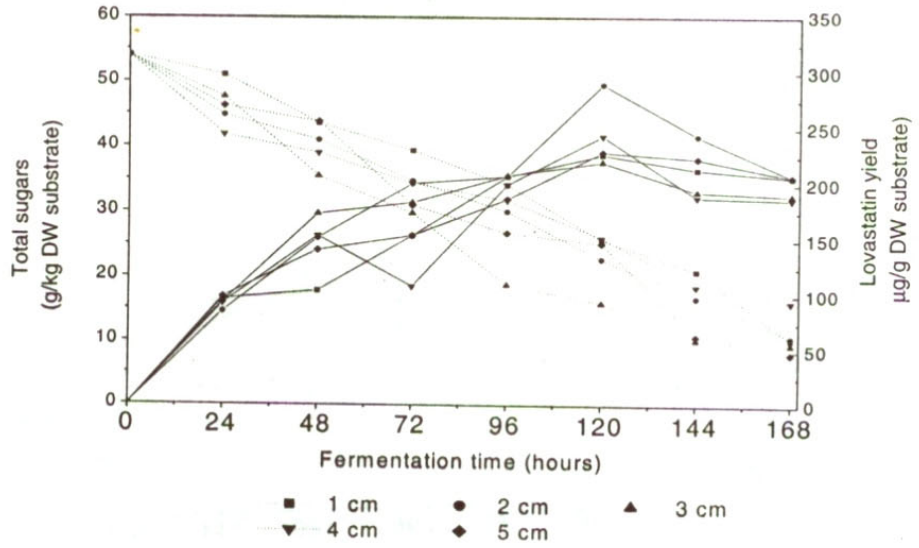
d. Effect of inoculum size: Optimization of inoculum size (spores/ml) is necessary in solid state fermentation because less amount of spores leads to insufficient biomass where as higher amount of spore concentration leads to too much biomass and quick depletion of nutrients. Hence, attempts have been made in this experiment to optimize inoculum concentration and the results are presented Fig. 4.

The data revealed that inoculum size of $1x10^8$ spores/ml of *Aspergillus terreus* KLVB28 yielded highest amount (280.66 mg/g DW) of lovastatin at 120 hrs fermentation period, while the lowest yield was observed at inoculum size of $1x10^5$ spores/ml, producing 155 mg/g DW of lovastatin in carob pods at the same fermentation period. Thus, the present study reveals that an inoculum size of $1x10^8$ spores/ml is suitable for maximum yield of lovastatin with *A. terreus* KLVB28 strain by employing carob pod as a substrate. This optimum density of spore inoculum used in the study is in agreement with the findings of Szakacs *et al.* (1998) and Lopez *et al.* (2003).

e. Effect of particle size: The particle size of the substrate greatly influences the production of lovastatin under SSF process. The results on the optimization of particle size for lovastatin production with *A. terreus* KLVB28 strain on carob pods are presented in Fig. 5. Amongst different particle sizes tested, the substrates with 2mm particle size has yielded maximum of 252.14 mg/g DW lovastatin at 120 hr. of fermentation period, where as the lowest yield 162 mg/g DW was observed with particle size 4mm and above. The maximum production at 2mm particle size indicates that the substrate provides sufficient surface area for the fungus growth and adequate sugar diffusion (Roukas, 1994).

f. *Effect of bed depth:* The thickness of substrate layer under natural fermentation conditions affects growth of the organism involves during the fermentation. The results obtained in the present study (Fig. 6) indicate that bed depth has a role in SSF of the carob pods to produce lovastatin. The yield of lovastatin enhanced as the bed depth increases from 1cm to 2cm and thereafter it decreases by further increase in bed depth of

Fig.6 Effect of bed depth on the yield of Lovastatin (____) by *A.terreus* KLVB28 and residual sugar concentration (....) in solid state fermentation of carobpods.



substrate layer. The maximum yield of 289.63 mg/g DW of lovastain was obtained at 2cm bed depth, during 120 hrs. of fermentation period. Since the studies were conducted under static conditions, proper aeration and gaseous exchanges could be obtained for optimum fermentation at this 2cm bed level or height. At higher bed depths lower yields of lovastatin were observed, that might be attributed due to lower metabolic activity of the organism coupled with improper aeration, gaseous exchange as well as unsuitable temperature elevations have impaired the lovastatin yields.

CONCLUSIONS

The present study reveals that moisture level of 65%, pH of 4.5, temperature of 35°C, inoculum size of $1x10^8$ spores/ml, particle size of 2mm and bed depth of 2cm is suitable for the maximum production of lovastatin under SSF employing *A. terreus* KLVB28, using deseeded carob pods as substrate.

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