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CHARACTERIZATION OF PHYSICAL FACTORS FOR OPTIMUM LOVASTATIN PRODUCTION BY *Aspergillus terreus* KLVB28mu21 UNDER SOLID STATE FERMENTATION

PRABHAKAR M[1]. LINGAPPA K[*]. VIVEK BABU[1]. AMENA S. VISHALAKSHI N. AND MAHESH D.

Department of Microbiology, Gulbarga University, Gulbarga- 585106 Karnataka, India. [1] Associate scientist, ITC Corp R&D, Bangalore, Karnataka, India. *Corresponding author E-mail- lingappak_micro@rediffmail.com

ABSTRACT

Lovastatin a hypocholesterolemic agent, competitively inhibits the rate limiting enzyme of cholesterol biosynthesis 3-hydroxy-3-glutaryl glutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the reduction of HMG-CoA to mevalonate during cholesterol biosynthesis. We studied lovastatin production by *Aspergillus terreus* KLVB28mu21 strain employing Solid State Fermentation of wheat bran. Highest yield (1110 g/gm of DW of substrate) of lovastatin was obtained at moisture content 65%, pH 5.5, temperature 30^0C, inoculum size $1x10^8$ spores mL^{-1} and bed depth 2cm.

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KEY WORDS: Characterization, physical, lovastatin, Solid State Fermentation, *A. terreus*.

INTRODUCTION

Lovastatin (mevinolin or monocolinK) a hypocholestromic agent, compitativly inhibit the rate limiting enzyme 3-hydroxy-3-methyl glutaryl co enzyme A (HMG-CoA) reductase which catalizes the reduction of HMG-CoA to mevalonate during cholesterol biosynthesis (1,2,3). This natural statin was the first fungal secondary metabolite to obtain approval from the US food and drug administration in August 1987 (4,5) in addition to this, lovastatin has clear evidence of benefit on stroke (6) and it also shows in-vivo tumor suppression by in habiting the synthesis of non sterol isoprenoid compounds (7). Lovastatin is produced by *Monascus* species (8), *Aspergillus terreus* (9), *Pencillium* species (10) and *Pleurotus* (11). Because of the hypocholesterolemic and hypolipaemic properties of lovastatin, search for efficient producers and perfection of improved production process are interest. To date the production of lovastatin under SSF usually carried out using defatted soya, sweet sorghum pulp, carob pods and wheat bran.

In the present work we have studied effect of several physical parameters like initial moisture, initial pH, incubation temperature, inoculums size and bed depth of the substrate (wheat bran) to develop a defined culture conditions for high production of lovastatin under Solid state fermentation.

MATERIALS AND METHODS:

The *A. terreus* KLVB28 strain was isolated from local soil and screened for lovastatin production through the *neurospora crass* bioassay method (12). Which is mutated with 0.5% of EMS for 5min and used for the lovastatin production under Solid State Fermentation (SSF). Spore suspension ($1x10^8$ spore mL^{-1}) of *A. terreus* KLVB28mu21 was prepared from seven day old culture grown on potato dextrose agar (PDA) slants by adding 10mL sterile distilled water containing 0.01% of Tween 80. The wheat bran was collected from local market with particle ranging from 2mm-5mm and dried in an oven at 60 ^{0}C for 3hrs thus prepared wheat bran (20g) was autoclaved in 250mL flasks at 121 ^{0}C for 20 min, cooled to room temperature, inoculated with 1mL of spore suspension and thoroughly mixed by gentle tapping. The static culture were incubated at 35 ^{0}C in slanting position for seven days

The fermentation process parameters studied for SSF were initial moisture, initial pH, incubation temperature, inoculums volume and bed depth. Once a given parameter was optimized, it was kept constant at that level while varying the other parameters individually. A set of conical flask, each containing 20g of substrate was moistened with distilled water ranging from 20-75% (v/w). Substrate having 65% of moisture level was studied at different initial pH levels ranging from 4.0-7.0. Wheat

bran with 65% moisture level and initial pH of 5.5 was incubated at different temperature ranging from 20^0C-50^0C. Substrate with 65% moisture content at pH 5.5 was inoculated with inocula having 10^5-10^{10} spore mL^{-1} and incubated at 30^0C for seven days in slanting position. Keeping moisture, pH, incubation temperature and inoculums at optimum levels, the bed depth was examined at 1-5cm.

Samples from fermented moldy substrate were withdrawn aseptically at 24h intervals for a period of 168h and analyzed for lovastatin as per (13), where in 1gm of moldy substrate was extracted with 10mL of acetonitrile and 0.5mL phosphoric acid. The extract was mixed on an orbital shaker for 60 min at 120 rpm and centrifuged at 5000 rpm. Supernatant was analyzed for lovastatin at 238nm, using UV-Vis spectrophotometer calibrated against known standards of lovastatin (12). The residual sugar in the fermentation medium was estimated by phenol sulphuric method (14). The observations were recorded for three replications and the mean values alone are reported.

RESULT AND DISCUSSION

Wheat bran served as good encourage medium for *A. terreus* KLVB28mu21, indicated by the formation of enhanced arial mycelium on the substrate. It also provided good substrate porosity for growth. There are many factors with affect maximum yield of lovastatin in SSF.

The moisture content of the substrate is one of the most important factor in SSF (15). The moisture level in SSF processes, which vary between 30 to 80%, here a marked effect on growth kinetics. The optimum moisture content for growth and substrate utilization is between 40 to 70%, but depends upon the organism and substrate used for cultivation (16). Employing the A. terreus KLVB28mu21 on wheat bran lovastatin production and sugar utilization were enhanced with increase in moisture content up to 65%, beyond which it declined (Fig. 1). Highest yield of lovastatin 730 µg/gm DW of substrate was obtained at a moisture level of 65%. Higher moisture level decreased porosity and reduced oxygen transfer (17). The results are in agreement with the result of szakaes (14), who reported maximum lovastatin production in sweet sorghum pulp and wheat bran at 75% moisture level. The hyphal mode of fungal growth and their good tolerance to low water activity (Aw) and high osmotic pressure conditions make it efficient and competitive in natural micro-flora for bioconversion of solid substrates.

Fig 1. Effect of moisture content on the yield of lovastatin by *A. terreus* KLVB28mu21 on wheat bran

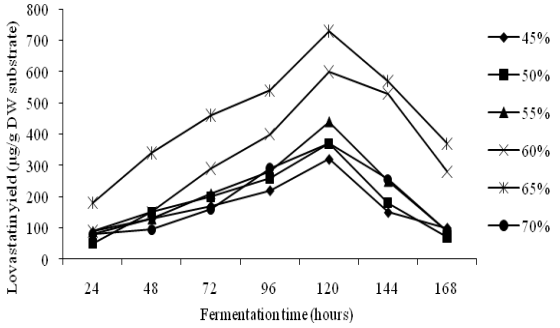
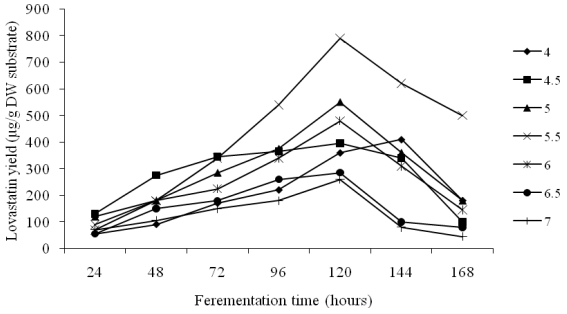


Fig 2. Effect of initial pH on the yield of lovastatin by *A. terreus* KLVB28mu21 on wheat bran



The production of lovastatin is greatly influenced by the initial pH of the substrate. szakaes (14) reported maximum production of lovastatin in sweet sorghum pulp at pH 6.2 and Valera (7) observed maximum lovastatin production at pH 5 using *A. flavipes* in wheat bran. In the present study, however, the most significant level of growth and production by pH ranging between 4.0 to 6.5. The effect of initial pH on wheat bran fermentation is presented in Fig 2. The lovastatin production was increased with increased in the pH of substrate up to 5.5 and thereafter it decreased. Maximum lovastatin production (790 µg/gm DW of substrate) was obtained at pH 5.5 at 120h fermentation time.

The effect of temperature on lovastatin production is shown in Fig 3. The lovastatin production increased with increase in

fermentation temperature from 20-30^{0}C and decreased above 30^{0}C (900 µg/gm DW of substrate) after 120h of fermentation, although appreciable amount of the enzyme production occurred at temperatures ranging between 30^{0}C. In contrast the temperature for lovastatin production was 25^{0}C on wheat bran and sweet sorghum pulp (14). Hajjaji (2) reported 35^{0}C as optimum in defined medium under submerged fermentation. Our observations are in agreement with the results of Hajjaji (2) Temperature is the most important physical variable affecting SSF (18), so we optimized the temperature to increase lovastatin production. Moreover, when strain was cultured at 40-45^{0}C, the medium dried fast and spores did not develop after 40^{0}C. So the optimum temperature was considered as 30^{0}C.

Fig 3. Effect of incubation temperature on the yield of lovastatin by *A. terreus* KLVB28mu21 on wheat bran

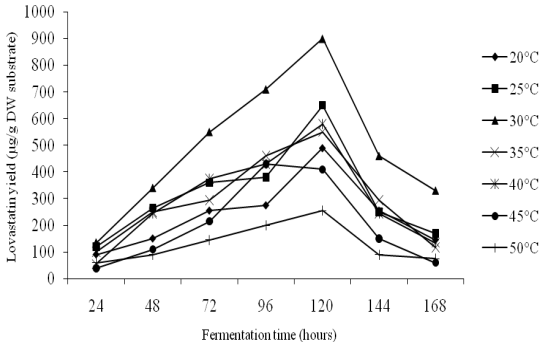
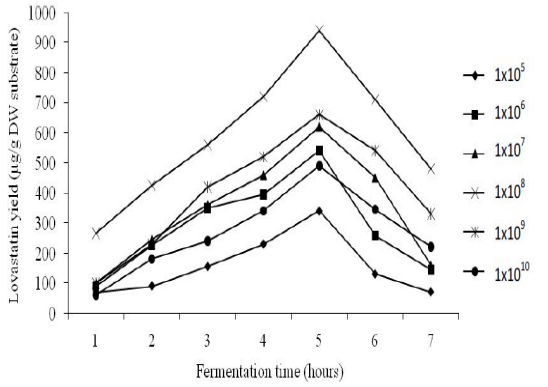


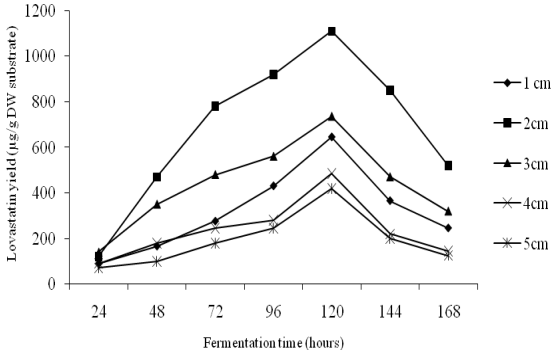
Fig 4. Effect of incubation temperature on the yield of lovastatin by *A. terreus* KLVB28mu21 on wheat bran



Optimization of inoculums size (spores mL^{-1}) is necessary in SSF because too few spores lead to insufficient biomass whereas too many spores lead to over production of biomass leading to quick depletion of nutrients. Hence attempts were made to optimize the titre value of the inoculum (Fig. 4). An inoculums size of 1x10^8 spores mL^{-1} yielded the highest lovastatin (940 µg/gm DW of substrate) after 120h fermentation, while the lowest activity was observed with inoculum size 1x105 spores mL^{-1} producing 340 µg/gm DW of substrate of lovastatin. The optimum density of spores inoculum used in the study was in good agreement with the findings of Lopez (19) and Szakacs (14) with good yield.

The bed depth has role in SSF of the wheat bran to produce lovastatin in Fig. 5. The thickness of substrate layer under natural fermentation conditions affects growth of the organism during fermentation. The yield of lovastatin enhanced as the bed depth increased from 1cm to 2cm and thereafter it decreased with increased bed depth. The maximum yield of 110 µg/gm DW of substrate of lovastatin was obtained at 2cm bed depth, during 120h fermentation period. Since the studies were conducted under static conditions, proper aeration and gaseous exchange could be obtained for optimum fermentation at this 2cm bed level/height. At higher bed depths lower yields of lovastatin were observed perhaps due to lower metabolic activity of the organism caused by reduced aeration.

Fig 5. Effect of bed depth on the yield of lovastatin by *A. terreus* KLVB28mu21 on wheat bran



Present study reveals that moisture level of 65%, pH 5.5, temperature 30^{0}C, inoculum 1x10^8 spores mL^{-1} and bed depth of 2cm is suitable for

the optimized production of lovastatin under SSF employing *A. terreus* KLVB28mu21 using wheat bran as substrate as substrate. Moreover, industrial metabolites were often produced in cheaper media such as Banana waste (20), sweet Sorghum (Szakacs 1998) *etc*. with higher yields. The wheat bran we screened is readily available and cheap in our country. Thus, our metabolite could be produced with higher yield easily and economically.

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References

1. Albert AW., Chen J., Kuroun G., Hunt V., Huff J., Hoffman C., et al. (1980). Mevinolin: A highly potent competitive inhibitor of hydroxylmethyl glutaryl coenzyme A reductase and cholesterol lowering agent. Proceedings of the national Academy of sciences of the united states of America 77(7): 3957.
2. Hajjaji H., Niedberger P. and Duboc. (2001). Lovastatin biosynthesis by Aspergellus terreus in a chemically definrd medium. Applied and Environmental microbiology 67(6): 2596.
3. Lingappa K. and Vivek Babu CS. (2005). Production of lovastatin by solid state fermentation of carob (Ceratonia siliqua) pods using *Aspergillus terreus* KLVB28. Indian journal of Microbiology 45(4): 283.
4. Tobert JA. (2003). Lovastatin and beyond: The history of the HMG-CoA reductase inhibitors. Nature Review Drug Discovery 2(7): 517.
5. Manjoni M. and Rollini M. (2002). Biosynthesis and biotechnological production of statin by filamentous fungi and applications of these cholesterol lowering drugs. Applied Microbiology and Biotechnology 58(5): 555.
6. Hebert PR., Gazion JM., Chan KS. and Henneken. (1997). Cholesterol lowering with statin drugs, risk of stroke and total mortality-an overview of randomized trails. Journal of American Medical Association 278: 313.
7. Valera HR., Gomes J., Laxmi S., Gururaj R., Suryanarayan S., Kumar D. (2005). Lovastatin production by SSF using *Aspergillus flavipes*. Enzymes Microb Techol 37: 521.
8. Endo A. (1979). Monacolin K, anew hypocholesterolemic agent produced by a Monscus species. Journal of Antibiotics 32: 852.
9. Albert AW. (1990). Lovastatin and simvastatin –inhibitors of HMG-CoA reductase and cholesterol-biosynthesis. Cardiology 77: 14.
10. Endo A., Kuroda M. and Ysujjita Y. (1976). ML-236A, ML-236B and ML-236 C, new inhibitors of cholesterogenesis produced by Penicillium citrinium. Journal of Antibiotecs. 29: 1346.
11. Gunde-Cimerman N., Friedrich J., Cimerman A. and Benicki. (1993). Screening of fungi for the production of an inhibitor of HMG-CoA reductase: Production of mevinolin by the genus *Pleurotus*. FEMS Microbiol Letter 111: 203.
12. Lingappa K., Vivek Babu CS., Siddalingeshwar KG. and Pramod T. (2004). Isolation, screening and rapid confirmation of lovastatin producing strains of Aspergillus terreus. Indian Journal of Microbiology 44: 137.
13. Morovjan G., Szakacs G. and Fekete J. (1997). Monitoring of selected metabolites and biotransformation products from fermentation broths by high performance liquid chromatography. Journal of chromatography 763: 165.
14. Szakacs G., Morovjan G. and Tengerdy RP. (1998). Production of lovastatin by a wild strain of Aspergillus terreus. Biotechnology letter 20: 411.
15. Lonsane BK., Gildyal NP., Budiatman S. and Ramakrishna SV. (1985). Engineering aspects of solid state fermentation. Enzyme microbial technol 7: 258.
16. Raimbault M. (1998). General and microbiological aspects of solid state fermentation. Electronic Journal of biotechnology 3: 174.
17. Zadrazil F. and Burnet H. (1981). Investigations on physical parameters on important for solid state fermentation of straw by white rot fungi. European journal of applied microbiology 11: 183.
18. Krishnna C. (2005). Solid state fermentation systems-an overview. Crit. Rev. Biotechnology 25: 1.

19. Lopez JLC. PEREZ jas. Sevilla JMF. Fernandez FGA. Grima EW. And Chistin y. (2003). Production of lovastatin by *Aspergillus terreus:* effects of the C:N ration and the principal nutrient on growth and metabolite production. Enzyme microbial technology 33: 270.
20. Ranganathan R. and Swaminathan K. (2005). Growth and amylase production by *Aspergillus oryzae* during solid state fermentation using banana waste as substrate. Journal of environmental biology 26(4): 265.